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SEXUAL BEHAVIOR AND MALE MATE CHOICE IN THE SPOTTED HYENA, CROCUTA CROCUTA

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Micaela Szykman

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SEXUAL BEHAVIOR AND MALE MATE CHOICE IN THE SPOTTED HYENA, CROCUTA CROCUTA

By

Micaela Szykman

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

SEXUAL BEHAVIOR AND MALE MATE CHOICE IN THE SPOTTED HYENA, CROCUTA CROCUTA

By

Micaela Szykman

Although female animals tend to be choosier than males with respect to their mate preferences, sexual selection theory predicts that males should also be selective when female fecundity varies. In this dissertation, I inquired whether male mate choice occurs in the spotted hyena (*Crocuta crocuta*). Although male hyenas do not invest heavily in reproduction in this species, reproductive success among female spotted hyenas varies greatly on the basis of social rank. Therefore, if males can assess the reproductive values of females, then sexual selection theory predicts that male hyenas should be highly selective in their choice of mates. Here, I looked for evidence of male mate choice in *Crocuta* by determining whether males interact differently with potential mating partners, and whether variation in these interaction patterns is correlated with male

To assess male choice of females, I calculated association indices for all male-female pairs, and analyzed rates of affiliative behavior directed by males toward females, in a group of free-living hyenas in Kenya. Males initiate most affiliative interactions with females, and males associate most closely with females that are likeliest to be fertile. High- and middle-ranking males associate most closely with high-ranking females, and some individual males pursue a strategy of long-term associations to acquire mates. Using molecular markers to determine paternity of cubs, I found that sires associate more closely with the mothers of those cubs than do non-sires, particularly during the last months before conception. These association data indicate that male spotted hyenas possess the ability to assess variation in female reproductive value, and they prefer females likeliest to maximize male reproductive success.

Masculinization of the female genitalia and female social dominance appear to influence sexual behavior in this species. Courtship behaviors in *Crocuta* are unique among mammals and reflect extreme ambivalence on the part of the male, who appears to be motivated by conflicting desires to approach the female and flee from her. Copulation provides a unique physical challenge for the male hyena, such that he must insert his erect penis into the female's forward-facing phallus, which remains flaccid during mating.

I also investigated the occurrence and function of male aggression directed toward females in this species. Male hyenas rarely direct aggression toward adult females, as females are socially dominant to all immigrant males in the clan. Most aggression directed by adult immigrant males against females occurs when coalitions of two or more males attack a single adult female, who responds by defending herself and fighting back. Daily attack rates vary significantly with female reproductive state, and the highest rates of male attack on females are observed when females are most likely to conceive. The adaptive significance of male aggression against females in this species is unknown, but my results are most consistent with predictions of an hypothesis suggesting that male aggression may function to inform the female about some aspect of male fitness.

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Images in this dissertation are presented in color.

Chapter 1

GENERAL INTRODUCTION

This dissertation describes intersexual association patterns and sexual behavior, and examines male mate choice in a gregarious mammal, the spotted hyena (*Crocuta crocuta*). Spotted hyenas possess many unusual behavioral and morphological traits that make them fascinating subjects for the study of sexual behavior. For example, female social dominance in spotted hyena groups creates a social environment that is unique among mammals, and influences male hyena courtship and mating behavior. Furthermore, high variation among female hyenas, with respect to their reproductive value, suggests that sexual selection may act in an unexpected way in this promiscuous species in which males provide no parental care, and in which dominant females are assumed to make final decisions regarding mating. In this introductory chapter, I set the stage for the study of male mate choice in *Crocuta* and explain why this peculiar animal is an appropriate subject for the study of this phenomenon.

In 1871, Darwin proposed the concept of sexual selection as a possible explanation for the existence of intraspecific sex differences in behavior and morphology, such as dimorphic body size and extravagant male ornaments. These traits could not be explained by natural selection because they did not improve survival, but they did appear to Darwin to improve mating success. Darwin (1871) identified two components of sexual selection: intrasexual and

intersexual selection. Intrasexual selection involves direct competition for mates among conspecifics of the same sex. Intersexual selection is mediated by choice and influences the likelihood that a given individual will be chosen as a mate by conspecifics of the opposite sex. Intrasexual selection is ubiquitous among animals and is most often characterized by a struggle between males for the possession of females (Darwin 1871; Clutton-Brock et al. 1982; Smuts 1987b). During intersexual selection, females typically have more opportunities than do males to choose a particular mating partner among many potential mates (Andersson 1982; Smuts 1987b). Thus in nature most intrasexual selection involves male-male competition and most intersexual selection involves female choice of mates.

The differential operation of these two modes of sexual selection in the two sexes was originally attributed to the highly unequal investments made by males and females in individual gametes (Parker et al. 1972). Anisogamy, the unequal size of male and female gametes, sets the stage for sexual conflict. In many species, females invest far more heavily than do males in the production of offspring, if for no other reason than because ova, in contrast to sperm, are generally laden with nutrients for early support of the embryonic offspring (Parker et al. 1972). As males compete for access to the most fecund females, females should be very careful in their choice of mating partners (Parker et al. 1972).

To show that these sex-specific differences in behavior result in differences in the potential rate of reproduction, it is necessary to obtain accurate data documenting differential reproductive success in males and females. The

potential rate of reproduction by males and females is measured as the maximum number of independent offspring that parents can produce per unit time (Clutton-Brock and Vincent 1991). In a classic study, Bateman (1948) observed that reproductive success was more variable in male *Drosophila* than in females. He determined that this variation existed because males could increase their reproductive success by increasing the number of females with which they mated, whereas females maintained the same reproductive success regardless of the number of matings they secured. That is, females were not limited by the number of mates they could attract but by other factors, including the number of mature eggs they possessed.

Trivers (1972) extended the idea of unequal reproductive investment by the two sexes beyond gamete production to encompass the contributions made by males and females in the form of parental care. He defined parental investment as "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring (p.139)." Parental investment may thus include providing nutrition to the female prior to offspring production in addition to the more common parental role of feeding and protecting offspring. Whether sexual selection acts via male-male competition or female choice of mates depends on limitations imposed by differential parental investment. Theory predicts that the sex investing more time and energy in parental care should become a limiting resource. Individuals of the non-limiting sex will necessarily compete for individuals of the limited sex. The limited sex

then has the opportunity to choose among competing prospective mates. Thus, the relative investment of each parent determines the extent to which animals compete for members of the limited sex (Thornhill and Gwynne 1986). Among mammals, in which obligate maternal care occurs with lactation, parental investment is greater for the female than the male, so theory predicts that sexual selection should operate in mammals via male-male competition and female choice. In fact, these patterns are typical of many ungulates (e.g. red deer, *Cervus elaphus*: Clutton-Brock et al. 1982), carnivores (e.g. sea lions, *Otaria byronia*: Campagna and LeBoeuf 1988) and primates (e.g. baboons, *Papio cynocephalus*: Altmann et al. 1988).

Examples of extended maternal care abound in the literature and are not limited to mammals. Many female birds are sole caretakers of their broods and are well known for their careful selection of males with long tails, colorful plumage or elaborate bowers (e.g. widowbirds, *Euplectes progne*: Andersson 1982; bowerbirds, *Ptilonorhynchus violaceus*: Borgia 1985; finches, *Carpodacus mexicanus*: Hill 1990). In species where males provide the majority of parental care, parental investment theory (Trivers 1972) predicts female-female competition over mates and male choosiness during mate choice (Petrie 1983; Oring et al. 1991; Andersson 1994). This theory may be limited in its practical application, since it fails to predict the circumstances under which competition or choice by both males and females should occur.

In addition to affecting potential reproductive rates of the two sexes, differential parental investment in offspring can also have an effect on the

operational sex ratio of a population. The operational sex ratio (OSR) is defined as the ratio of sexually active males to sexually receptive females in a population at any given time (Emlen and Oring 1977). Factors affecting OSR include biased overall sex ratios, reproductive longevity, differential mortality rates, migration schedules, reproductive synchrony, physiological constraints on reproduction. and the spatial and temporal distribution of resources in the environment (Emlen and Oring 1977; Kvarnemo and Ahnesjo 1996). OSR was first presented by Emlen and Oring (1977) as a concept useful in elucidating the intensity of sexual selection in a population. They argued that OSR provides "an empirical measure of the degree of monopolizability of mates," and OSR can therefore be used in ecology as a predictor of mating systems. In addition, the greater the degree of monopolization, the more intense the intrasexual selection will be, and the greater the resulting variance in reproductive success within the competing sex. Thus, OSR can be used to predict the intensity of intrasexual competition and the direction of sexual selection (Gwynne 1981; Kvarnemo et al. 1995; Kvarnemo and Ahnesjo 1996). For example, one would expect to find intense male-male competition when operational sex ratios are strongly male biased.

Theoretical developments since 1977 elaborate on the use of operational sex ratio as a predictor of sex roles in reproductive behavior by including sex differences in variance in mate quality as an additional factor influencing the direction of sexual selection (Gwynne 1981; Parker 1983; Petrie 1983; Owens and Thompson 1994). Specifically, these authors suggest that OSR can be used to predict the direction of intrasexual selection, or which sex will be competing for

mates, but argue that OSR does not predict extent or direction of mate choice. These authors propose that high variance in the quality of one sex promotes increased mate choice behavior in the opposite sex. Mate quality may be measured in a variety of ways, including measuring traits like female size or by calculating lifetime reproductive success. For example, female size is strongly correlated with fecundity in animals of indeterminate growth such as insects (Gwynne 1981) and fish (Côte and Hunte 1989). Thus, if female body size is a normally distributed trait within a population, we should expect increased mate choice by males, who should have the ability to evaluate the reproductive potential of females, as predicted by body size.

Gibson and Langen (1996) agree that variance in mate quality will influence mate choice. However, they further suggest that costs of searching for mates might influence the level of optimal choosiness (for more on search theory and costs of searching, see Real 1990). For example, male thirteen-lined ground squirrels, *Spermophilis tridecemlineatus*, use information on female reproductive condition to guide their future search activities. That is, males invest more time searching for females that they anticipate will be in estrus the next day (Schwagmeyer 1995). Owens and Thompson (1994) incorporate search costs, including predation risk and physiological constraints on reproduction, into their model describing the behaviors which should be optimized during mate choice, specifically the trade-off between reproductive rate and mate quality. As noted above, the direction of sexual selection can be predicted by different potential reproductive rates in males and females (Clutton-Brock and Vincent 1991), since

OSRs are biased in favor of the sex with higher reproductive rates. However, when there is sufficiently greater variation in mate quality among the sex with the lower potential reproductive rate, the sex with the higher potential mating rate should be the more choosy sex (Owens and Thompson 1994).

To summarize, competition between males is expected to occur when (1) male parental investment involves little or nothing more than the initial contribution of sperm, (2) males have higher potential reproductive rates than do females, and (3) operational sex ratios are male-biased, especially under conditions of highly monopolizable resources (food and/or mates). Intersexual selection via female mate choice is expected when (1) females invest more than males in parental care, (2) females have lower reproductive rates than do males, (3) OSR is male-biased and resources are monopolizable, and (4) there is variation in male mate quality.

Although male-male competition and female mate choice are the most common expressions of the operation of sexual selection in animals, Darwin (1871) himself recognized that rare cases occur in nature in which males might have the opportunity to choose the most attractive females from an array of mates. Indeed, male mate preferences have been documented in nature in studies reporting that males prefer older, more experienced mates (Burley and Moran 1979), younger mates who are likely to be virgins (Forsberg 1987), larger, more fecund females (Gwynne 1981; Verrell 1985; Verrell 1989; Berglund and Rosenqvist 1993; Verrell 1995), females who exhibit better parental care (Verrell 1990; Solomon 1993), and females with higher reproductive potential (Berger

1989; Schwagmeyer and Parker 1990; Schwagmeyer 1995). In general, male mate choice is expected when males invest heavily in reproduction, when females are synchronous in their sexual receptivity, or when there is high variation in the quality of potential mates (Gwynne 1981; Parker 1983; Owens and Thompson 1994).

Male investment in reproduction may take the form of increased parental care, as in some monogamous mammals (e.g. carnivores: Moehlman 1989; rodents: Solomon 1993; primates: Tardiff 1997) and many species of fishes (e.g. Gross and Shine 1981; Berglund and Rosenqvist 1993). Alternatively, some male insects, e.g. Mormon crickets (*Anabrus simplex*), transfer personal, nonrenewable nutrients, in the form of a large spermatophore or nuptial gift, to a female during copulation, at a significant cost to themselves (Gwynne 1981; Forsberg 1987). In such cases, males are expected to discriminate among females with respect to their allocation of expensive ejaculate or spermatophore, and indeed they do (Gwynne 1981). High male investment now limits the number of matings possible for males and generates opportunities for eliciting female-female competition. Finally, variation in female quality promotes male mate choice.

Some males prefer mates based on their previous reproductive success and on their future reproductive potential. In bison (*Bison bison*), males identify, and choose to mate with, more fertile females (Berger 1989). Support for male discrimination in bison is presented by Komers and colleagues (1994) who show that males are more likely to tend females when the females are close to estrus,

as indicated by low progesterone levels. In feral pigeons (*Columba livia*), both males and females prefer more experienced mates, but only up to a certain age when great age appears to become a liability offsetting the benefit of greater experience (Burley and Moran 1979). These forms of male mate choice require that males be capable of evaluating females. Tests of the hypothesis that variation in female quality promotes male mate choice must show that (a) females vary with respect to their reproductive potential, (b) females of varying potential are simultaneously available, (c) males do not copulate at random, and (d) when given simultaneous presentation, males will selectively mate with females promoting higher fitness, not just with as many females as possible (Parker 1983; Petrie 1983). In this dissertation, I attempted to test these predictions to address the hypothesis that variation in female quality promotes selective mate choice in male spotted hyenas.

Spotted hyenas exhibit many unusual behavioral and morphological traits that are reversed from mammalian norms. They live in matriarchal, femaledominated societies in which the females are more aggressive, and approximately 10% larger, than males (Kruuk 1972; Frank 1986b). In addition, females exhibit highly masculinized external genitalia complete with pseudoscrotum and a fully erectile phallus (Matthews 1939; Neaves et al. 1980). Although spotted hyenas are monomorphic in appearance, and although they exhibit patterns reversed from mammalian norms with respect to social dominance, hyenas nevertheless exhibit many of the same sex differences as those seen in virtually all other mammals. For example, females are responsible

for all parental care, and males take the traditional, active role in courtship (Kruuk 1972; Frank 1986b). Despite several long-term field studies on this species conducted by excellent scientists (Kruuk 1972; Frank 1986b; Frank 1986a; Mills 1990), little is known either about sexual behavior of hyenas in the wild, or about features of mate choice in this species.

The present study was initiated after Holekamp and colleagues (1996) determined that female spotted hyenas do, in fact, vary with respect to their reproductive potential. Similarly, intrasexual variation in reproductive success is also associated with social rank in many other gregarious mammals (e.g. primates: Altmann et al. 1988: ungulates: Clutton-Brock et al. 1988: carnivores: Creel et al. 1992). Crocuta clans are structured by rigid dominance hierarchies, and an individual's position in this hierarchy determines its access to food and other resources (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986a; Henschel and Skinner 1987). In this species, female social rank significantly influences several aspects of female reproductive success. High-ranking female hyenas begin breeding at younger ages, are more frequently able to support pregnancy and lactation concurrently, experience shorter intervals between litters, and their offspring are more likely to survive to adulthood than are those of lower-ranking females (Frank et al. 1995a; Holekamp et al. 1996). Considering the significant variation observed in female reproductive success in this species, if males can assess reproductive value of females, then sexual selection theory predicts that male hyenas should be highly selective in their choice of mates. Male behavior patterns that increase the probability that a particular male will mate with a

certain individual female are indicative of male mate choice (Halliday 1983). Here, I looked for evidence of male mate choice in *Crocuta* by determining whether males interact differently with potential mating partners, and whether variation in these interaction patterns is correlated with male reproductive success. My specific goals were (1) to examine predictions of modern sexual selection theory in a socially role-reversed mammal, the spotted hyena, in which females vary significantly in their quality as mates, (2) to further elucidate the mating system of this species, and (3) to describe male-female interactions not previously reported in the literature, and to attempt to elucidate the functional significance of a subset of these.

To achieve these goals, I utilized demographic and behavioral data collected over 11 years by researchers on the Mara Hyena Project, initiated in 1988 by Drs. Kay Holekamp and Laura Smale. Although I personally spent over two consecutive years in the field between June 1997 and August 1999 collecting detailed accounts of courtship and sexual behavior, sample sizes for these behaviors were greatly increased by supplementing my own data with archived data from several years. I also made use of paternity data painstakingly generated by many workers from several labs in two countries (Libants et al. 2000; Engh et al. 2002; S.M. Funk and A.L. Engh, unpublished data). Linking patterns of male *Crocuta* behavior and mate choice with male reproductive success could not have been achieved without these essential paternity data. Therefore, I have elected to use the term "we" throughout the following three data chapters to indicate the collaborative nature of this intensive study.

The first step I took toward evaluating male mate choice was to examine basic association patterns among male and female spotted hyenas. My goal in Chapter 2 of this dissertation was therefore to test the hypotheses that, because females vary with respect to their quality as mates, (1) male hyenas should preferentially associate with high-quality females, and (2) male preferences should additionally be affected by female reproductive state. I predicted that male hyenas should associate most closely with high-ranking females and with females most likely to be fertile. Interactions between pairs of adult males and females were observed intensively in our study population of free-living hyenas in Kenya during a 7-year period. During this time, multiple females were known to conceive around the same time, creating opportunities for male choice. Behavioral data extracted from focal animal surveys (Altmann 1974) indicated that males initiate and maintain most associations with females. I subsequently evaluated the strength of male-female associations by calculating association indices (using the twice-weight index of association: Cairns and Schwager 1987) for all male-female pairs. Results unambiguously showed that male hyenas associate most closely with high-ranking females and with females that are likeliest to be fertile. High- and middle-ranking males associate most closely with high-ranking females, but low-ranking males associate equally closely with females in all rank categories, suggesting that males in different rank categories may be using different reproductive strategies to attract females or obtain them as mates. Using molecular markers to determine paternity of cubs born during the study period, I found that sires associate more closely with the mothers of

those cubs than do non-sires, particularly during the last months before conception. These association data indicate that male spotted hyenas do indeed exhibit selective mate choice, and that they prefer females likeliest to maximize male reproductive success, thus confirming both hypotheses addressed in this chapter. This chapter was published recently in *Behavioral Ecology and Sociobiology*, so future reference in Chapters 3, 4, and 5 to the association data reported in Chapter 2 will cite the published manuscript of Szykman et al. (2001).

The association data provide only partial insight into individual male behavior and decision-making regarding mate choice, so I next examined courtship and mating behavior in Crocuta in more detail in Chapter 3. The goals of this chapter were to document male and female sexual behavior, including courtship and mating, not previously described in the literature, and to evaluate the hypothesis that male affiliative behaviors reflect mate choice. I predicted that males would exhibit more affiliative behaviors towards high-ranking females and toward females closest to their time of known conception. Furthermore, given a choice among multiple females of various ranks and in various reproductive states, a male should actively choose to maintain associations with females likeliest to maximize his reproductive success. In Chapter 3, I first describe courtship and mating behavior in Crocuta, and then test the predictions outlined above. I found that males direct most affiliative behaviors toward females approaching conception, and that male decisions about staying with an individual female are influenced by her social rank and her reproductive state, measured as time to conception.

Finally, I investigated the occurrence and function of rare male aggression directed toward females in this species. My specific goals in Chapter 4 were to describe the conditions under which male attacks on females occur, shed light on the mechanisms mediating male aggression toward females, and address possible adaptive functions of this unusual behavior. Male hyenas rarely direct aggression toward adult females, as females are larger and socially dominant to all immigrant males in the clan (Kruuk 1972; Frank 1986b; this study). Most aggression directed by adult males against females occurs when coalitions of two or more males attack a single adult female, who responds by defending herself and sometimes fighting back (Kruuk 1972; this study). Kruuk (1972) described these coalitionary attacks by males against females, and referred to them as 'baiting'. Although Kruuk doubted that these attacks were related to sexual behavior in hyenas, I included analyses of baiting in this dissertation because my data suggest these attacks may play some role in hyena courtship. Specifically, daily baiting rates vary significantly with female reproductive state, and the highest rates of male attacks on females are observed during the period when she is most likely to conceive. The adaptive significance of male aggression against females in this species remains unknown, but my results are most consistent with an hypothesis suggesting that male aggression informs females about male fitness.

The dissertation concludes with a discussion and integration of the three data chapters. In summary, sexual behavior in the spotted hyena is unique among mammals. Masculinization of female genitalia and social dominance of

females appear to influence the structure of male courtship and other sexual behaviors in this species. Furthermore, my data suggest that male hyenas make choices about with which females to associate, and with whom they should ultimately mate, apparently based on the males' ability to assess variation in female reproductive value. The cues males use to make these assessments remain unknown, but I will speculate on these and other unanswered questions in my conclusions in Chapter 5.

Chapter 2

ASSOCIATION PATTERNS AMONG MALE AND FEMALE SPOTTED HYENAS REFLECT MALE MATE CHOICE

INTRODUCTION

Because female animals produce larger gametes and often invest more time and energy in parental care than do males, limited access to females usually limits male reproductive success (Parker et al. 1972; Trivers 1972; Andersson 1994). Females can, therefore, generally be highly selective in their choice of mates. However, Darwin (1871) recognized that males might also sometimes have opportunities to choose the most attractive females from an array of mates. Indeed, in various animal species, male preferences have been documented for older females with more experience (Burley and Moran 1979), younger females likely to be virgins (Forsberg 1987), larger and more fecund females (Gwynne 1981; Verrell 1985; Sargent et al. 1986; Verrell 1989; Berglund and Rosenqvist 1993; Verrell 1995), females with higher reproductive potential (Berger 1989; Schwagmeyer and Parker 1990; Schwagmeyer 1995), and females exhibiting superior parental care (Verrell 1990; Solomon 1993). In general, male mate choice is expected when males invest heavily in reproduction or when there is high variation in the guality of potential mates (Gwynne 1981; Parker 1983; Owens and Thompson 1994). Here we inquire whether male mate choice occurs in a long-lived social mammal, the spotted hyena. Although males do not invest

heavily in reproduction in this species, females vary greatly in their ability to produce surviving offspring.

Spotted hyenas are gregarious carnivores that live in social groups called clans. Each clan contains one to several matrilines of adult females and their offspring, as well as one to several adult immigrant males. *Crocuta* 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 hour to hour. Although associations among clan members shift frequently, clans are nevertheless rigidly structured by hierarchical rank relationships (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986a; Holekamp and Smale 1990; Holekamp and Smale 1993), and an individual's social rank determines its priority of access to food (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986a). Female hyenas are philopatric, but all males disperse to new clans sometime after reaching reproductive maturity at two years of age (Smale et al. 1997). Adult females are socially dominant to all adult males not born in the clan (Kruuk 1972; Smale et al. 1993).

Female reproductive success varies enormously with social rank in this species (Frank et al. 1995a; Holekamp et al. 1996). In fact, reproductive skew among female *Crocuta* is greater than that observed among females of any other plural-breeding mammalian species for which comparative data are available (Holekamp and Smale 2000). High-ranking female hyenas begin breeding at younger ages, are more frequently able to support pregnancy and lactation concurrently, experience shorter intervals between litters, and their offspring are more likely to survive to adulthood than are those of lower-ranking females

(Holekamp et al. 1996). Considering the significant variation observed in female reproductive success in this species, if males can assess reproductive value of clan females, then sexual selection theory predicts that male hyenas should be highly selective in their choice of mates, preferring high- over low-ranking females. Male behavior patterns that serve to increase the chances that a particular female will mate with a certain individual male are indicative of mate choice (Halliday 1983; Bercovitch 1991). Here we looked for evidence of male mate choice in *Crocuta* by evaluating variation in interactions observed between males and their potential mating partners, and then inquiring whether variation in these interaction patterns is correlated with male reproductive success. One mechanism by which a male might be able to increase his chances of mating with a particular female is the development of an affiliative relationship with her (Smuts 1983; Bercovitch 1995). In this paper, we present data addressing the questions of whether male hyenas prefer to associate with high-ranking females, and whether male preferences vary with female reproductive state.

METHODS

Study animals and observational techniques

We conducted this study in the Talek area of the Masai Mara National Reserve, Kenya. This is an area of open, rolling grasslands grazed year round by large concentrations of several different ungulate species. The subject population was one large *Crocuta* clan inhabiting a home range of approximately 65 km². We

monitored Talek hyenas continuously from June 1988 to June 1995. Throughout this period, we conducted behavioral observations of Talek hyenas 23 to 31 days per month, except during April 1991, when observers were only present on 14 days. Between 1988 and 1995, the Talek study clan varied in size from 50 to 70 individuals, consisting on average of 12 adult immigrant males (range: 6-20) and 20 adult females (range: 16-28) and their juvenile offspring. We identified all hyenas in the Talek clan individually by their unique spot patterns, and we determined their sex from the dimorphic glans morphology of the erect phallus (Frank et al. 1990).

We established mother-offspring relations on the basis of regular nursing associations. We assigned birth dates to litters by estimating cub ages when they were first observed above ground at natal or communal dens. Cub ages could be estimated to ± 7 days based on the pelage, size, and other aspects of cub appearance and behavior. We determined social ranks of individual hyenas based on outcomes of several thousand dyadic agonistic interactions during which one individual exhibited submissive behavior to the other, but regardless of whether or not the submissive behavior was elicited by aggression from the social partner (Holekamp and Smale 1990; Smale et al. 1993). Adult females and adult immigrant males were ranked in separate intrasexual dominance hierarchies. By convention we assigned the highest-ranking (alpha) individual in each hierarchy a rank position of one. Only immigrant males present in the clan for longer than 6 months were included in the male hierarchy. For some analyses we divided each intrasexual dominance hierarchy into equal thirds: high, middle,
and low ranks representing ranks 1-7, 8-14, and \geq 15, respectively.

We conducted daily behavioral observations from vehicles between 0530 and 0900 h and between 1700 and 2000 h. During the study period, we observed hyenas for a total of 4407.8 hours during 15,353 observation sessions. We initiated an observation session when we first drove up to one or more hyenas separated from others by at least 200 m. An observation session ended either when all hyenas moved out of sight, for example into bushes, or when we drove on to a new location. We identified all individual hyenas present in each session, and we excluded from analyses any sessions in which one or more unidentified hyenas were present. We located hyenas while driving daily circuits around the study clan's home range, visiting the area's high points, and scanning with binoculars to sample all parts of the home range every day for presence of subgroups of hyenas. We then visited each subgroup to determine its composition. We also conducted multiple 30-minute focal animal surveys (Altmann 1974) on each of 15 adult male and 23 adult female hyenas to compare rates at which males and females initiated and maintained associations with opposite-sex conspecifics. In each survey we calculated the rate at which the focal animal approached opposite-sex conspecifics, and we also calculated the percent total time in each survey the focal animal spent following opposite-sex conspecifics. An approach was scored when a focal hyena moved to within 1 m of another individual. The focal animal was considered to be following another hyena in any observation minute during which it walked behind another individual moving in the same direction. An hourly approach rate was calculated for each

focal animal as [(# of approaches / # minutes observed with opposite-sex conspecific) * 60]. Percent time spent following was calculated as [(# of min spent following / # min observed with opposite-sex conspecific) * 100]. We calculated mean values for each individual observed during multiple surveys.

Evaluation of male-female associations

We calculated an association index (AI) for each male-female pair using the twice-weight index of association (Cairns and Schwager 1987):

$$(A + B)_{together} / [(A_{without B}) + (B_{without A}) + (A + B)_{together}]$$

where $(A_{without B})$ represents the number of observation sessions in which female A was observed but male B was not present, $(B_{without A})$ represents the number of observation sessions in which male B was present but female A was absent, and $[(A + B)_{together}]$ represents the number of sessions in which both female A and male B were present. Als were calculated only for females observed throughout complete reproductive cycles within the study period. A complete reproductive cycle began at the time of conception of one litter in which at least one cub survived to weaning, and ended at the conception of the subsequent litter. For cases in which paternity of cubs was known, we examined association patterns relative to the date of a female's conception. In other analyses, we divided each complete reproductive cycle into five reproductive states as follows.

Pregnancy (P): The duration of pregnancy in *Crocuta* is 110 days (Schneider 1926; Kruuk 1972), so we calculated conception dates by subtracting 110 days from estimated birth dates to obtain conception dates that are accurate

to \pm 7 days. However, since the extent of variation in gestation length in this species is not currently known, for analyses in which we focused on male-female interactions around the time of conception, we considered a female's "fertile period" as her date of conception \pm 1 week.

Lactation (L1, L2, L3): The lactation period began with the birth of a litter and ended with its weaning. Weaning conflicts and cessation of nursing indicated when cubs were weaned (Holekamp et al. 1996). Weaning conflicts between cubs and their mothers are easily observed in this species due to the characteristic begging behavior and loud whining exhibited by cubs at this time. We recorded all weaning conflicts in field notes as critical incidents ("alloccurrence" sampling, Altmann 1974). In determining weaning dates, we searched all field notes for observations of nursing behavior when mother and cub were found together. If mother and cub were not found together frequently after the last observed nursing bout, the weaning date was identified as being midway between the last nursing bout and the next sighting of mother and cub together without nursing (Holekamp et al. 1996). All weaning dates used in these analyses were accurate to within ± 10 days. Lactation periods varied in length among Talek females from 7 to 21 months (Holekamp et al. 1996; Holekamp and Smale 2000). Therefore, to better compare association patterns among females with different lactation period lengths, we divided the total period of lactation for each female into three intervals of equal length, represented as L1, L2, and L3.

Other (O): Females assigned to this condition had weaned one litter but had not yet conceived their next litter. Little is known about the estrous cycle of

the spotted hyena. Matthews (1939) and Lindeque (1981) suggest that estrous cycles in this species recur every 14 days. However, there are no apparent morphological indicators of estrus in female spotted hyenas as there are, for example, in many cercopithecine primates (reviewed in Melnick and Pearl 1987), and copulations are rarely observed in *Crocuta*. Although most females weaned one litter weeks or months before conceiving another, females of all social ranks were occasionally observed to conceive while still nursing a previous litter (Holekamp et al. 1996). Here, females known to be pregnant while still nursing a previous litter weeks).

The ability of male hyenas to access or monopolize fertile females should vary with the degree to which estrous cycles are synchronized among clan females. Therefore we estimated the extent to which female fertile periods overlapped during the study period, by assuming that such overlap was possible if a particular conception occurred within 2 weeks of conception in another female. We considered asynchronous conceptions to be those occurring more than 2 weeks before or after any other conception known to occur during the study period.

Most rank positions in the female dominance hierarchy were occupied by multiple individuals during the course of this study. We therefore calculated overall mean association indices for all rank positions in the female hierarchy by summing AIs across all female reproductive states for all females holding each rank, and dividing by the total number of AIs. We then compared mean AIs of male-female dyads among social rank categories and among female

reproductive states. Using only associations observed between 1990 and 1995 for a subset of females bearing litters during this period, we also compared female associations with known sires to those with males known not to have sired their litters.

In addition to calculating association indices, we also calculated the mean number of males present with each female and compared these values among female reproductive states and categories of female social rank. Finally, we were interested in comparing male-female associations in *Crocuta* with the malefemale "consortships" observed in other mammals. Therefore we determined the percent of all sessions in which each male was observed during which he was found alone with a particular female. To do this, we counted the number of sessions a pair was seen alone together, without any other hyenas present, divided that value by the total number of sessions the male was observed, then multiplied the result by 100. We did this for each month-long interval surrounding conception of the female's litter.

Paternity determination and statistical analysis

We used microsatellite markers identified from *Crocuta* DNA to determine male parentage in the Talek clan. Between 1990 and 1995, we collected blood from 185 Talek hyenas. Sampling was conducted after individuals were anaesthetized with Telazol (2.5 mg/kg) delivered from a CO₂-powered rifle in a lightweight plastic syringe. We then immediately extracted DNA from blood using Puregene kits (Gentra Systems, Inc.), and stored the extracted DNA in liquid nitrogen.

We considered all adult males present in the clan at the time of conception as potential sires. Paternity assignments were based on 13 microsatellite markers described elsewhere (Libants et al. 2000; S.M. Funk and A.L. Engh, unpublished data). Paternity was determined using three approaches. First, males were excluded as potential fathers if their genotypes were inconsistent with those of offspring; that is, if each putative father-cub pair did not share an allele at every locus surveyed. Paternity was also evaluated by testing significance of pairwise relatedness (r_{xy}: Queller and Goodnight 1989), and by a maximum-likelihood analysis (program 'Cervus') which compared likelihoods of paternity for all non-excluded males (e.g., most likely fathers, second most likely fathers, etc., Marshall et al. 1998). A male was only assigned as the sire of a particular cub if exclusion probability (Chakraborty et al. 1988) exceeded 0.99, if the calculated r_{xy} value did not differ significantly from 0.5, and if the maximum likelihood method assigned paternity at a confidence of 95%. If two of the three conditions were not satisfied, then we considered paternity unresolved. A more comprehensive analysis of paternity in the Talek clan is presented by Engh et al. (2002).

We analyzed Association Index data using 1-way, 2-way and repeated measures analysis of variance (ANOVA). For analyses in which we compared Als within a male-female dyad across months, we treated Als generated monthly by each pair as repeated measures. We evaluated relationships among smaller subsets of the data that were not normally distributed, using Mann-Whitney U tests. We calculated correlation coefficients (Spearman's R) to ascertain whether

mean male-female AIs varied with female social rank. We presented mean values as $\overline{X} \pm SE$. We considered differences between groups to be statistically significant when P<0.05.

RESULTS

Effects of social rank and female reproductive state

We calculated overall Als for 40 immigrant males observed together with 19 adult females. Association indices ranged from 0 to 1, with a mean of 0.062 \pm 0.002. Social ranks ranged from 1 to 20 for females and from 1 to 24 for males. Overall mean Als between males and females varied significantly with female social rank, with males associating more closely with high-ranking than with low-ranking females (Figure 2.1; Spearman rank correlation: R_s=-0.760, N=19 ranks, P<0.001).

We then examined the effects of female reproductive state and rank category on patterns of association. Females were observed over 40 complete reproductive cycles (\overline{X} =2.1 ± 0.1 cycles per female). We observed individual females in each reproductive state for 0-844.6 hours: \overline{X}_P =66.2 ± 14.8, \overline{X}_{L1} =120.5 ± 22.1, \overline{X}_{L2} =84.8 ± 18.2, \overline{X}_{L3} =56.0 ± 12.4, \overline{X}_O =24.5 ± 6.8 hours per female. Maximum Als reached 1.00 in all states except 'P' and 'L1', which had the lowest maximum Al values of 0.368 and 0.500, respectively. Males not only associated most closely with the highest-ranking females when females were grouped into three rank categories (Figure 2.2; ANOVA: F_{2.2650}=58.325, P<0.001), but also



Figure 2.1. Mean (\pm SE) intersexual association indices plotted as a function of female social rank. By convention, the highest rank possible is one. Each point represents the mean AI for all females who held a particular rank with all males observed with each female while she held that rank. N=2665 associating pairs involving 19 females and 40 males associating during all female reproductive states.



Figure 2.2. Mean association indices for male-female pairs measured across all female reproductive states and grouped by female social rank. Female reproductive states are as follows: P=pregnancy; L1, L2, L3=first, second, and third trimesters, respectively, of the lactation period; O=other states, when adult females are neither pregnant nor lactating. Rank categories: high=ranks 1-7, middle=ranks 8-14, low=ranks 15 and higher. Sample sizes are as in Figure 2.1.

with females that were most likely to be fertile, during states 'L3' and 'O' (Figure 2.2; $F_{4,2650}$ =12.959, P<0.001). Moreover, there was a significant interaction between female rank and reproductive state, indicating that males associated most closely with high-ranking females at times when these females were most likely to be fertile (Figure 2.2; 2-way ANOVA: $F_{8,2650}$ =7.565, P<0.001).

Male social rank also influenced intersexual association patterns in hyenas. High-ranking males associated more closely with high-ranking females than did lower-ranking males (Figure 2.3; 2-way ANOVA: $F_{4,2656}$ =5.150, P<0.001). In fact, both high-ranking males and high-ranking females associated more closely overall with members of the opposite sex than did lower-ranking individuals (males: $F_{2,2656}$ =45.458, P<0.001; females: $F_{2,2656}$ =18.447, P<0.001). Post-hoc tests revealed that both high- and middle-ranking males associated most closely with high-ranking females (Figures 2.3A and B), while low-ranking males tended to associate more or less equally with females in all three rank categories (Figure 2.3C; high-ranking males: $F_{2,1341}$ =34.917, P<0.001; middleranking males: $F_{2,1059}$ =15.767, P<0.001; low-ranking males: $F_{2,256}$ =1.291, P=0.28).

A female Talek hyena may travel alone, with kin, with a single adult male, or with a sizeable entourage of males. The average number of males present with an individual female varied with female reproductive state (Figure 2.4; ANOVA: $F_{4,4793}$ =10.347, P<0.001) but not with female social rank ($F_{2,4795}$ =1.229, P=0.29). The greatest number of males associated with females during the

Figure 2.3. Mean (\pm SE) association indices between females in each rank category and (A) high-ranking males, (B) middle-ranking males, and (C) low-ranking males. Rank categories are as in Figure 2.2 and are the same for both sexes. Sample sizes are as in Figure 2.1.









Figure 2.4. Mean number of males observed per session with each female across all female reproductive states. N=4798 sessions for which 19 females were observed with 40 males. Bars sharing same letters represent means not significantly different from one another as determined by Tukey's post-hoc comparisons.

reproductive states 'L3' and 'O', when females were most likely to conceive, but also during pregnancy (Tukey's post-hoc comparisons).

To evaluate the roles played by adults of each sex in initiating associations, we calculated rates of behavior performed during 346 focal animal surveys on 15 adult males, and during 127 focal animal surveys on 23 adult females, for which at least one adult member of the opposite sex was present (males: \overline{X} =23.1 ± 4.3 focal animal surveys / individual; females: \overline{X} =5.5 ± 0.7 focal animal surveys / individual). Males approached females at significantly higher rates than vice versa, and in fact, females hardly ever approached males (males: 0.036 ± 0.007 approaches / hour; females: 0.002 ± 0.001 approaches / hour; Mann-Whitney U test: U_1 =16.000, P<0.001). Similarly, females rarely followed males, and males spent a significantly greater percent of their focal animal time following females than females spent following males (males: 8.6 ± 1.7 % of their time spent following females; females: $0.5 \pm 0.2\%$ of their time spent following males; Mann-Whitney U test: U₁=10.000, P<0.001). Thus, associations between male and female spotted hyenas appeared to be initiated and maintained almost exclusively by males.

Reproductive synchrony among Talek females

Dates of 105 fertile periods were known during the study period from 1988 to 1995 for 35 female hyenas, so we evaluated reproductive synchrony by measuring overlap among these fertile periods (\overline{X} =2.7 ± 0.2 fertile periods per female). We found that 56.3% of fertile periods occurred when no other Talek

female was concurrently fertile (Figure 2.5). Although up to five females were observed to be fertile concurrently during the study period, this occurred only once, and on average, only 1.6 ± 0.1 females were fertile concurrently. Thus, reproductive synchrony among Talek females was generally low throughout the study period. (Addendum. In August of 1998, there was a 2-week period when the fertile periods of nine females overlapped. This was highly unusual, and was possibly due to atypical ecological conditions experienced as a consequence of an El Niño event.)

Comparing sires with non-sires

We next contrasted intersexual associations involving males who sired cubs with those of males who failed to sire offspring. We examined AIs during month-long intervals surrounding the conception of a litter for which the father was known via paternity analysis for a subset of eight females, each of which had a complete reproductive cycle preceding that conception. Males known to sire the offspring of these females during these particular reproductive cycles associated significantly more closely with their mates than did other males (Figure 2.6A; 2-way repeated measures ANOVA: $F_{1,86}$ =14.064, P<0.001), particularly near the time of conception (Figure 2.6A; $F_{12,86}$ =1.961, P=0.038). Finally, we evaluated a male's ability to maintain exclusive access to a particular female by examining the percent of all observation sessions in which he was observed during which he was found alone with that female. Sires spent significantly more time alone with their future mates (Figure 2.6B; 2-way repeated measures ANOVA:



Figure 2.5. Synchrony among 105 fertile periods of 35 females observed between 1988 and 1995. Sample sizes represent the number of 2-week intervals during which females were concurrently fertile.

Figure 2.6. (A) Association indices for eight females with the eight males who sired (solid bars) their litters conceived during interval '0' compared with Als for these same females with 15 males present concurrently who did not sire (open bars) their litters. Interval '0' represents the date of conception \pm 2 weeks. Remaining intervals represent month-long periods surrounding the date of conception. (B) Percent of observation sessions during which sires and non-sires were found alone with females, calculated as [(the number of sessions in which the pair was found alone/the total number of sessions in which that male was observed)*100].



в



 $F_{1,86}$ =6.501, P=0.012) than did males failing to sire offspring, particularly during the months immediately preceding conception (Figure 2.6B; $F_{12,86}$ =1.857, P=0.05).

DISCUSSION

Our association data indicate that male hyenas appear to assess relative reproductive value of individual females, and that they may allocate their own reproductive effort among clan females based on this assessment. Male hyenas associate most closely, and in the greatest numbers, with females that are most likely to be fertile. Males of other mammalian species similarly prefer to associate with females when they are most likely to conceive (e.g. baboons, Papio cynocephalus: Seyfarth 1978a, b; Smuts 1985; bison,: Komers et al. 1994). Since neither visual nor acoustic signals emitted by female hyenas appear to vary with their reproductive condition, males presumably evaluate female reproductive state using olfactory cues. Although large numbers of males associated with females during pregnancy as well as during periods of maximal fertility (Figure 2.4), exclusive associations between females and sires were not observed after the first month of gestation (Figure 2.6B). Since females and sires do not remain together until cubs are born, exclusive male-female associations in Crocuta apparently do not function to reduce infanticide, as occurs in some primates (e.g. Sterck et al. 1997). Instead male hyenas may be unable to detect the early stages of pregnancy in females, or they may associate with pregnant

females to increase the probability of mating with them in the future. Studies of various primates show that a male can increase his chances of mating with a particular female by associating with her, even when she is not fertile (e.g. Smuts 1983; Smuts 1985; Hill 1987; Bercovitch 1995), and this may also hold true in *Crocuta*. Male hyenas might also continue to associate with their mates for a short time after conception to guard them from rival males, as occurs in lions (*Panthera leo*: Packer and Pusey 1982).

Like spotted hyenas, lions are polygynous carnivores in which individual males consort with fertile females. Reproduction is highly synchronous within lion prides, and several females may be in estrus at once (Schaller 1972; Bertram 1975). Although rival males are more likely to be found near a consorting pair when availability of other estrous females is low, consorting lion pairs are found alone in approximately fifty percent of sightings (Schaller 1972; Packer and Pusey 1982). By contrast, consorting hyena pairs are found alone in less than five percent of sightings (Figure 2.6B), so it appears that male hyenas may be far less successful than male lions at monopolizing access to fertile females. Male lions often 'herd' females away from other pride members (Packer and Pusey 1982), but female *Crocuta* are dominant to males, so male behavior does not constrain female movement in this species.

In our study population reproductive synchrony among females is low (Figure 2.5), as compared to lions, for example, where 50-75% of females in a pride may be concurrently in estrus (Schaller 1972). Low reproductive synchrony among female hyenas should theoretically permit male hyenas to associate

closely with fertile low-ranking females without foregoing mating opportunities with higher-ranking females. Although we found that males associated with dominant females to the greatest extent at times when those females were most likely to be fertile, most males preferred high-ranking females during all stages of the female reproductive cycle. This supports the hypothesis that males actively choose among females, and indicates that males attempt to maximize their chances of mating with females having the greatest potential to produce surviving offspring, and thus also to maximize male reproductive success. Similarly, in many primates, males show mating preferences for high-ranking females (reviewed in Robinson 1982; Silk and Boyd 1983).

In groups of canids and social mongooses, the highest-ranking individuals of each sex prefer the highest-ranking opposite-sex individual as their resting and grooming partners, closest associates, and mates (Rasa 1977; Derix and van Hooff 1995). In the current study, social rank of both male and female hyenas influenced intersexual patterns of association. Both high- and middle-ranking males associated most closely with the highest-ranking females, but low-ranking males failed to exhibit a preference for higher-ranking females. Low-ranking males may thus either be less adept than high-ranking males at assessing reproductive value of clan females, or low- and high-ranking males may utilize different reproductive strategies.

Our comparison of association patterns of sires and non-sires suggests that, when a male hyena invests time with one prospective mate, this may pay off in terms of increasing his chances of mating with that female. However, our data

also suggest that time spent with one particular female represents a substantial investment that might compromise the male's ability to explore alternative reproductive possibilities. Sires spend more of their time in exclusive isolation with their future mates than do non-sires, particularly during the last months before conception. One obvious benefit to associating with a female in isolation is exclusive access to that female when she is ready to mate. If a male associating with a particular female can keep her away from rivals, he has a greater chance of siring that female's offspring. Immigrant male Crocuta rarely fight among themselves (Kruuk 1972; Frank 1986b), but the fission-fusion nature of hyena society may permit males to use physical separation with prospective mates as an effective alternative to combat, as also occurs in lions (Packer and Pusev 1982). Interestingly, although female hyenas in the current study were highly attractive to large numbers of males when females were likeliest to conceive (Figure 2.4), known sires were able to maintain exclusive access to females relatively often during these periods of maximal fertility (Figure 2.6B).

Close and exclusive intersexual associations were related to paternity in the current study, but the factors causing these patterns remain unknown. That is, a male might make himself more attractive to a particular female by spending more time with her, and thus enhancing his own chances of siring her cubs. Alternatively, a male might spend more time with a female because she has already chosen him to sire her cubs. Thus our data do not rule out the possibility that close intersexual associations in *Crocuta* reflect mate choice by females as well as by males. Indeed, female hyenas make exceptionally heavy and

prolonged investment in each of their cubs (Hofer and East 1993; Hofer and East 1995; Holekamp et al. 1996), and receive no help from males in rearing young. suggesting that females should be highly selective in their choice of mates. Nevertheless, the reproductive performance of high-ranking females is far superior to that of low-ranking females (Holekamp et al. 1996), generating extreme variance in female reproductive success, and thus mate quality, as a function of social rank. Therefore, male *Crocuta* ought to be choosy, and our data are consistent with this hypothesis. In fact, we found that male-female associations in this species are almost exclusively initiated and maintained by males. In contrast, intersexual consortships in other social carnivores and in primates frequently involve a cooperative effort by both members of the pair to maintain proximity (Stern and Smith 1984; Small 1990). In these other mammals consorting pairs are observed to copulate, and consorting males father most offspring (Packer and Pusey 1982; Bercovitch 1987; Small 1990). We rarely observe hyenas mating so we don't yet know to what extent copulation frequency in Crocuta can be predicted by male-female association patterns (but see Chapter 3). However, unusually close intersexual associations are correlated with paternity in *Crocuta*, as they are in other gregarious mammals.

Finally, our data allow us to contrast male-female association patterns in *Crocuta* with patterns previously documented for female-female and motheroffspring associations in this same species. Using the same AI calculations as those utilized here, Holekamp et al. (1997) showed that association patterns among adult female *Crocuta* are strongly influenced by both kinship and rank.

Adult females associate more closely with their adult female kin than with unrelated adult females, and high-ranking mother-offspring pairs associate more tightly than do low-ranking dyads. When we compare absolute values of association between that earlier study and this one, we find that, on average, adult males associate with adult females at about the same levels as adult females associate with other unrelated adult females. Furthermore, males siring litters associate with their mates almost as closely as do mothers with their own offspring, with Als over twice as high as those of males failing to sire those cubs. Although male-male association patterns have not yet been examined in this species, our results suggest that adult males may form their closest associations with the females with whom they eventually mate.

Chapter 3

COURTSHIP AND MATING IN FREE-LIVING SPOTTED HYENAS

INTRODUCTION

Courtship and mating are two aspects of sexual behavior required for the successful fusion of egg and sperm in many animals, and the peculiar courtship and mating behaviors of the spotted hyena are the focus of this chapter. The functions of animal courtship behavior are both numerous and diverse. Courtship behaviors may function to facilitate recognition by potential mates of species and sex (Littlejohn and Loftus-Hills 1968; Ptacek 2000), or to synchronize physiological and behavioral processes necessary for fusion of gametes (Crews 1975; Verrell 1997). Courtship behaviors may also indicate mate quality, and permit opportunities for mate choice (Clutton-Brock et al. 1982; Bradbury and Gibson 1983; Karino 1995; Hoikkala et al. 1998). Finally, courtship may help break down barriers that prevent mates from coming together to copulate, particularly in solitary or aggressive species (Caro 1994). Courtship and mating in carnivorous animals can be potentially dangerous, since these behaviors bring together mates that are aggressive and well armed.

Mating systems in mammalian carnivores range from monogamous to highly polygynous or promiscuous, and successful mating is achieved with a variety of reproductive strategies (Schaller 1972; Ewer 1973; Clutton-Brock 1989;

Creel and Macdonald 1995). In general, mammalian mating systems are determined by the dispersion of resources and mates (Emlen and Oring 1977; Clutton-Brock 1989) and the degree of parental care required to maximize reproductive success for both sexes (Davies 1991). Male and female sexual strategies vary as access to, and competition over, mates differ between solitary and group-living species.

Whether solitary or gregarious, monogamous or polygynous, the challenges to achieving successful copulation among mammalian carnivores begin with finding a receptive conspecific and convincing this individual to consider mating. This latter task may require extensive courtship and moderate persuasion on the part of the male and various physiological and behavioral responses on the part of the female. The male may attempt to restrain the female by grabbing her from behind with his front paws and teeth at the time of copulation, as observed in most felids and mustelids (Schaller 1972; Ewer 1973; King 1989; Caro 1994). This behavior may serve to keep the female from biting the male, to elicit an immobilization response in the female similar to that seen in cubs when being carried by their mothers (Schaller 1972; Vargas and Anderson 1998), or to help the male orient his body properly for achievement of intromission (King 1989; Beaver 1992). Some males may injure or even kill females while trying to mate with them (e.g. elephant seals, *Mirouanga elaphus*: Cox and LeBoeuf 1977: LeBoeuf and Mesnick 1990).

Successful copulation requires physiological and behavioral coordination between males and females. Many females in the felid, mustelid, ursid,

herpestid, and procyonid families of carnivores are induced ovulators, and ovulation is triggered by repeated or lengthy bouts of copulation (Hayssen et al. 1993). For example, mating pairs of lions will copulate every 15-30 minutes for 2-4 days (Schaller 1972; Bygott et al. 1979; Packer and Pusey 1983). Canids are not induced ovulators, but mating pairs remain locked together in a 'copulatory' tie', which has been reported for all canid species in which copulation has been observed (Asa and Valdespino 1998). A copulatory tie or lock is a mechanical connection formed between the vagina and the penis which holds the mating pair together following penile insertion (Dewsbury 1972). In canids, this tie is caused by a swelling of the bulbis glandis, near the base of the penis (Beach and LeBoeuf 1967). Unlike the sexual behavior of many canids and felids, which has been studied extensively, little is known about the coordination of behavioral events required for successful copulation in hyenids. This chapter aims to elucidate sexual behavior in a peculiar gregarious member of the carnivore family Hyaenidae, the spotted hyena.

Spotted hyenas are particularly interesting subjects for the study of sexual behavior for two reasons. First, spotted hyenas live in female-dominated societies in which females are larger and more aggressive than males, and females are socially dominant to adult breeding males (Kruuk 1972; Smale et al. 1993). Second, female *Crocuta* possess unusually heavily 'masculinized' genitalia. The female's clitoris is elongated and fully erectile, and her labia are fused and filled with connective tissue, thus resembling male testes (Matthews 1939; Neaves et al. 1980; Hamilton et al. 1986). This unique female morphology

makes intromission by the male considerably more difficult than it is in other mammals, and also makes copulation by force physically impossible. These odd behavioral and physical characteristics exhibited by female spotted hyenas may force males to make extra efforts to convince females to mate with them, and pose several other unusual problems for male hyenas. First, the male must determine when a female is in estrus, even though it may be dangerous for him to approach her. Second, the male must attract a female and convince her that he is a suitable mating partner. Third, the male must insert his erect penis into the female's flaccid pseudo-penis to successfully achieve intromission.

Very little is known about courtship and mating in spotted hyenas, and even less is known about the underlying reproductive physiology, or even about the length of female estrous cycles. Grimpe (1916) reported that a captive female came into estrus 3 weeks after parturition when cubs were removed, and then again 2 weeks later, though no physical or behavioral indicators of estrus were mentioned. Matthews (1939) and Lindeque (1981) used histological analyses of the ovaries of wild hyenas to similarly conclude that female hyenas in the wild cycle approximately every 14 days. In other studies, estrous cycles in captive *Crocuta* have been reported as 21-60 days (Schneider 1926) and 45 days (Golding 1969). The duration of the estrous period, the length of time that a female hyena is receptive to sexual advances by the male, remains unknown.

Kruuk (1972) was the first to conduct long-term research on spotted hyenas in the wild. However, during almost four years of detailed observations of hyenas in the Serengeti National Park and Ngorongoro Conservation Area in

Tanzania, he only made 14 observations of mounting behavior, and only five of these appeared to culminate in intromission (Kruuk 1972). He reported that male hyenas are always afraid of, and subordinate to, females, and that males show little or no aggression toward females during mating. Therefore, Kruuk suggested that the female's cooperation is essential to the success of the copulation. He also observed that there appears to be a surprisingly high level of tolerance among rival males in this species, but he believed that only one of the several males present with a receptive female is likely to mate with her. Kruuk concluded that the "pair bond" is weak in *Crocuta*, and he observed no clear pattern of any one female associating with a particular male for an extended period near the time of conception. One goal of the present study was to replicate and elaborate on Kruuk's limited findings in regard to mating behavior in *Crocuta*.

Frank (1986b) described his own observations of male sexual behavior in wild spotted hyenas in Kenya, and preliminarily outlined the effects of social rank on reproduction. He divided male courtship into attendance behaviors and active courtship. Attendance behaviors included following and consorting with a female by a male. Active courtship behaviors, which were seen only in the context of male-female interactions, included three types of behaviors. First, the male behaved submissively by bobbing his head up and down as he slowly approached the female. Second, the male experienced an approach-avoidance conflict whereby any movement of the female would cause him to retreat, after which the male would resume his deliberate approach. Third, the male exhibited a unique behavior that consisted of extending his foreleg and crossing it over the

opposite foreleg (Frank 1986b). Frank maintained that any adult male might follow, consort with, or occasionally court a female, but that eventually the highest-ranking (alpha) male will exclude all other males using only mild threats, gain exclusive access to all receptive females, and mate with them. Frank concluded that spotted hyenas have a highly polygynous mating system, in which the alpha male sires a disproportionately large number of cubs (Frank 1986b).

Mills (1990) observed that sexual behavior by male spotted hyenas in the Kalahari was similar to that described by Kruuk (1972) and Frank (1986b). Kalahari clans are smaller than clans in East Africa and usually contain only one immigrant male who performs all observed matings (Mills 1990). Immigrant Kalahari males investigate, court, and attempt to mate with clan females, following the characteristic behavior patterns originally described by Kruuk (1972). Mills further suggested that persistence might be the key to male reproductive success, rather than intense male-male competition. Like Kruuk (1972) and Frank (1986b), Mills did not observe any overt aggression among males competing over direct access to a female. One of our goals in this chapter was to quantify the time and effort that males must invest to gain mating opportunities with receptive females.

Two recent reports from our research group indicate that *Crocuta* are best described as promiscuous (*sensu* Clutton-Brock 1989): males mate with multiple females, and there appears to be no lasting bond between individual males and females after mating has occurred (Engh et al. 2002; Szykman et al. 2001). Male-female association patterns indicate that male spotted hyenas prefer

females likeliest to maximize their own reproductive success (Szykman et al. 2001). Females have been observed mating with multiple (up to three) males during a single estrous period (over four consecutive days), and males have been observed mating with several different females over the course of several years (Engh et al. 2002). Both observations of mating and paternity tests based on microsatellite data confirm that males ranked 1-14 in the male hierarchy copulate with females, and that males ranked 1-11 sire cubs. Some litters (approximately 20%) are sired by multiple males (Engh et al. 2002).

Successful reproduction requires active participation by both sexes, and its occurrence is profoundly influenced by female sexual behavior. Female animals differ in the extent to which they attract mates and in how they respond to male sexual overtures. After attempting to quantify various characteristics of female mammals in estrus, Beach (1976) concluded that there are three different components of female sexuality. Attractivity represents the 'stimulus value' of the female to the male. This includes both behavioral and non-behavioral cues, which are influenced by ovarian hormones, and which can be measured by the male's interest in the female. These cues function to attract males to the female, and provide males with information about the female's reproductive status. Proceptivity reflects the female's attraction to the male and consists of appetitive activities directed by the female toward the male in response to stimuli received from the male. Proceptive behaviors indicate the female's motivation to mate, serve to bring the male and female into proximity, and arouse the male (and perhaps also the female) to facilitate successful copulation. The female's specific

behavioral responses that are 'necessary and sufficient' for successful copulation indicate female *receptivity*, the female's readiness to allow copulation. Although female sexual behavior in *Crocuta* has not previously been investigated with Beach's (1976) schema in mind, we will do so here.

We seek to add to existing knowledge about sexual behavior in *Crocuta* by presenting data collected during 11 years of observation of a free-ranging group of spotted hyenas in Kenya. In this chapter, we will describe and quantify sexual behavior in free-living spotted hyenas, including pre-copulatory affiliative behaviors that serve to bring the sexes into proximity, as well as the postures and actions involved in mating itself. We will examine rates of male affiliative behaviors toward females during the months surrounding conception, report detailed sequences of events from field observations of courtship and mounting, and document aspects of female sexual behavior not previously well-described in the literature.

METHODS

Study animals and observational techniques

Spotted hyenas are gregarious carnivores that live in social groups called clans. Each clan contains one to several matrilines of adult females and their offspring, as well as one to several immigrant adult males. Clans are rigidly structured by hierarchical rank relationships (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986b; Holekamp and Smale 1990; Mills 1990; Holekamp and Smale 1993), and

an individual's social rank determines its priority of access to food and other resources (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986b). Adult females are socially dominant to all adult males not born in the clan (Kruuk 1972: Smale et al. 1993). Female hyenas are philopatric, but all males disperse to new clans sometime after reaching reproductive maturity at 2 years of age (Smale et al. 1997). Natal males may remain in their natal clans up to 38 months after puberty (Smale et al. 1997), thus generating two types of adult males in every clan: adult natal males and immigrant males. Natal males differ significantly from immigrant males with respect to intrasexual aggressive behavior, intersexual courtship behavior, and plasma testosterone profiles (Holekamp and Smale 1998). Natal males are significantly more aggressive toward other adult males than are immigrants, are socially dominant to all immigrants, and are the winners of all fights with immigrants. Despite the fact that they outrank immigrant males, natal males court females at significantly lower rates than do immigrant males, and natal males have significantly lower plasma testosterone levels than do immigrant males, even when controlling for male age and body size (Holekamp and Smale 1998).

We conducted this study in the Talek area of the Masai Mara National Reserve, in southwest Kenya. This is an area of open, rolling grassland grazed year round by large concentrations of several different ungulate species. The subject population was one large *Crocuta* clan inhabiting a home range of approximately 65 km². We monitored Talek hyenas continuously from June, 1988 to August, 1999. Between 1988 and 1999, the Talek study clan varied in size

from 45 to 79 individuals, not including transient individuals present in the clan for less than 6 months. The clan consisted on average of 14 adult immigrant males (range: 5-16) and 19 adult females (range: 12-26) and their juvenile offspring. We identified each individual hyena in the Talek clan by its unique spot patterns, and we determined its sex from the dimorphic glans morphology of its erect phallus (Frank et al. 1990).

We established mother-offspring relations on the basis of regular nursing associations. We assigned birth dates to litters by estimating cub ages when they were first observed above ground at natal or communal dens. Cub ages could be estimated to \pm 7 days based on their pelage, size, and other aspects of cub appearance and behavior. We were then able to calculate conception dates by subtracting the average gestation period of 110 ± 1 days (Schneider 1926; Kruuk 1972; M.L. Weldele, pers. comm.) from estimated birthdates. We determined social ranks of individuals based on outcomes of several thousand dyadic agonistic interactions during which one individual exhibited appeasement behavior to the other, but regardless of whether or not the appeasement was elicited by aggression from the social partner (Holekamp and Smale 1990; Smale et al. 1993). Adult females and adult immigrant males were ranked in separate intrasexual dominance hierarchies. Only immigrant males present in the clan for longer than 6 months were included in the male hierarchy. By convention, we assigned the highest-ranking (alpha) individual in each hierarchy a rank position of 1.

We conducted behavioral observations from vehicles between 0530 and 0930 hours and between 1630 and 2000 hours. During the 11-yr study period, we observed hyenas for 8166 hours during a total of 39,600 observation sessions. Adult female and adult male hyenas were present together for 3477 hours during 7260 observation sessions. We initiated an observation session when we first drove up to one or more hyenas separated from other individuals or groups by at least 200 m. An observation session ended either when all hyenas moved out of sight, for example into bushes, or when we drove on to a new location. We identified all individual hyenas present in each session, and we excluded from analyses any sessions in which one or more unidentified hyenas were present. We located hyenas while driving daily circuits around the study clan's home range, visiting the area's high points, and scanning with binoculars to sample all parts of the home range every day for presence of subgroups of hyenas. We then visited each subgroup to determine its composition and identified all individual hyenas present. Note that some of the analyses presented below utilize a subset of the source data (e.g. data from only two of the 11 total years).

Operational definitions of monitored behaviors

We wanted to quantify male affiliative behavior toward females, and vice versa, so we recorded the following behaviors for all individuals as critical incidents (Altmann 1974) during interactions between males and females.

General affiliation

Approach. An individual hyena walks to within 1m of another hyena.

Follow. An individual hyena walks behind another hyena within 100m, and moving in the same direction.

Affiliative investigation

Sniff. An individual hyena approaches within 1m of another hyena, or its urine, puts its nose down, and inhales its scent. After sniffing an adult female or her urine, a male may display flehmen, a behavior exhibited by various mammals and characterized by an open mouth and retraction of the upper lip, often with head extended or elevated (Estes 1972).

Lick. An individual hyena makes contact with another hyena with its tongue.

Greet. Two hyenas stand head-to-tail and sniff each other's genitals. When both members of the pair lift their inside hind legs, this is considered a complete, or symmetrical, greeting. If only one individual lifts his/her hind leg, and the other individual sniffs without lifting his/her hind leg, this is considered an asymmetrical greeting (after East et al. 1993).

<u>Courtship</u>

Although we were uncertain about the exact functions the following behaviors fulfilled, we included them here as courtship behaviors because they were only exhibited by adult male spotted hyenas toward adult females, and they appeared to indicate a male's sexual interest in a particular female.
Approach-Avoid. A male walks within 10m of a female, who is usually lying down, and repeatedly alternates taking a few steps toward her, then retreating a few steps back. It is not necessary for the female to make any motion to prompt the male's retreat.

Present. A male stands in front of a female, at right angles to her, with his legs stiff and outstretched anteriorly and posteriorly, letting the female sniff his side, ventrum, and genitalia. The male often appears nervous and is very skittish as the female sniffs him.

Bowing display. A male approaches a female, stops, crosses one foreleg over the other, and lowers his head. Sometimes he licks one of his extended forelegs while they are crossed or rubs his face against his foreleg.

Paw ground. A male approaches a female and repeatedly scratches the ground in front of her with his forepaw.

Mating

The term mating, or mating behavior, as used throughout this study, encompasses the following three behaviors.

Mount. An adult male, positioned behind a standing female, lifts his forepaws up on top of the female's back or hindquarters. We defined a short mount as any mount lasting less than 60 s and a long mount as any mount lasting longer than 100 s.

Intromission. A male successfully inserts his penis into the female's urogenital canal. Observers can usually determine when intromission has

occurred (e.g. see Figure 3.4B). When intromission occurs during a mount, we refer to it as copulation.

Ejaculation. It is very difficult to determine when ejaculation has occurred in free-living hyenas, but we adopt here a suggestion proposed by S.E. Glickman and L. Koscia (pers. comm.) based on their observations of captive hyenas. That is, it appears likely that a male hyena has ejaculated once he has finished thrusting and remains mounted on the female with his chin and chest resting on her back for several minutes (see Figure 3.3C below). Therefore, when a mounted male was observed to lower his head and chest onto the female's back in the current study, we interpreted this to mean that ejaculation had occurred.

Female sexual behavior and responses to male affiliative behaviors

Largely because copulations are so seldom observed in free-living hyenas, little is known about female sexual behavior in *Crocuta*. Here, we measured *attractivity* of females using male-female association patterns and male affiliative behaviors directed at females. We considered approach of males by females, and following of males by females, as *proceptive* behavior in this species. We monitored female receptivity by recording female responses to male *affiliative* behavior. Three possible types of responses were recorded: neutral, positive, or negative.

Neutral. The female makes no motion in response to a male's advance. She may just lie or stand still and allow the male to sniff her, lick her, etc.

Positive. The female approaches or follows the male.

Negative. The female makes a threatening gesture towards the male, or lunges at, chases, or bites him.

For all male-female affiliative interactions observed between June 1997 and August 1999, we calculated daily rates of male affiliative behavior by dividing the number of times each behavior occurred within a pair by the total number of hours each male-female pair was observed together during each of 7 month-long intervals surrounding a female's known conception, and then multiplying this number by 24. Together, the two 2-week intervals on either side of the conception date represented month "zero". To be included in this analysis, it was necessary that the male and female members of the pair be observed together for at least 1 hour during each month-long interval. For these analyses, we divided male affiliative behaviors into three categories.

- A) We analyzed *approach* behavior alone in its own category.
- B) We grouped sniff, lick, and greet into a category designated as *investigatory* behaviors.
- C) We grouped approach-avoid, present, bowing display, and paw ground into the category of *courtship* behaviors.

We then examined rates of these behaviors in relation to female social rank and female age. Female social rank was divided into three categories: high, middle, and low rank categories represented ranks 1-7, 8-14, and \geq 15, respectively. Female age was similarly divided into three categories: young, middle, and old age classes represented ages 2-6, 7-11, and 12-16 years, respectively.

We defined a "mating session" as an observation session during which we observed any mounting behavior. We calculated daily mating rates by dividing the number of mating sessions observed in the morning (0500-0900 hours) and evening (1600-2000 hours) sessions by the total number of hours hyenas were watched during these periods, and then multiplying by 24. We recorded mount duration as the length of time (in seconds) a male remained mounted atop the female and inter-mount interval as the length of time that elapsed between long mounts. Matings were considered fertile (i.e. resulting in conception) when a litter was observed within 1 month of the predicted birth date. This represents an extremely liberal criterion for assessment of the fertility of a particular mating. We calculated a predicted birth date by adding 110 days (average gestation period) to the date on which we observed copulation or mounting.

We calculated rejection coefficients (RC), in the manner of Beach and LeBoeuf (1967), to determine whether females treated adult immigrant and adult natal males differently. RC was calculated as follows:

affiliative behaviors to which the female responded with aggression
total # affiliative behaviors exhibited by the male towards the female

Female aggressive responses included all levels of threat, from mild threat, through chase and intention movement to bite, to actual biting.

Paternity determination

We used microsatellite markers identified from *Crocuta* DNA to determine male parentage in the Talek clan. Between 1990 and 1999, we collected blood from

191 Talek hyenas. Sampling was conducted after individuals were anaesthetized with Telazol (2.5 mg/kg) delivered from a CO₂-powered rifle in a lightweight plastic syringe. We then immediately extracted DNA from blood using Puregene kits (Gentra Systems, Inc.), and stored the extracted DNA in liquid nitrogen.

We considered all adult males present in the clan at the time of conception as potential sires. Paternity assignments were based on 12 microsatellite markers described elsewhere (Libants et al. 2000; S.M. Funk and A.L. Engh, unpublished data). Briefly, we employed a maximum-likelihood approach (Thompson 1975; Meagher 1986) to assign paternity to cubs with known, genotyped mothers using the program CERVUS (Marshall et al. 1998). A male was considered the father of a cub when CERVUS assigned him as such with 95% confidence. A more comprehensive analysis of paternity in the Talek clan is presented by Engh et al. (2002).

Initiation of inter-sexual interactions

We next compared the behavior of male and female hyenas in mating vs. nonmating situations. To reiterate, we defined a mating session as an observation session during which we observed any mounting behavior. We designated observation sessions that did not contain mounting behavior, as 'other' sessions. For each mating pair, we calculated the rate at which each animal approached its partner, and we also calculated the percent total time during each mating session each animal spent following its partner. An hourly approach rate was calculated for each individual as [(# of approaches / # minutes observed during mating

session) * 60]. Percent time spent following was calculated as [(# of min spent following / # min observed during mating session) * 100]. We calculated mean values for individuals if they were observed during multiple mating sessions. Finally, we compared the rates of approach and following behavior by males and females during these mating sessions to those calculated in 'other' sessions, as described above.

Male-female associations

Using only litters for which sires could be identified by paternity analysis, we compared sires and non-sires with respect to their association patterns with females around the time these litters were conceived. Here, we simply recorded whether each male was present with the female during each consecutive session in which the female was observed, starting 3 mos before conception and ending 1 mo after conception. Numbers of consecutive sessions with observations varied considerably among females for this 4-month-long period, but this analysis was useful nonetheless to inquire whether sires appeared to be engaging in any sort of consortship or mate-guarding behavior.

Male choice: logistic regression

For data collected between June 1997 and June 1999, we recorded two additional aspects of male behavior to evaluate male mate choice. First, when a male sniffed an individual female during an observation session, we subsequently recorded whether or not that male stayed with the female he had

sniffed (sniff-and-stay) until they disappeared out of sight or until observers left that session. Second, when a male showed a clear preference for one individual female over others during an observation session, the male was recorded as *choosing* that female. A male was determined to be exhibiting a clear preference (choice) for a particular female when he repeatedly courted and/or followed a single female throughout a session in which at least one other adult female was present. Both of these male decision-making behaviors were recorded as dichotomous values: sniff-and-stay vs. sniff-and-go or choice vs. no choice, and these were mutually exclusive.

We then determined which female characteristics influenced male choices. We used logistic regression to determine which independent variables influenced the value of eachh dichotomous dependent variable (Legendre and Legendre 1998; Hosmer and Lemeshow 2000). Female predictor, or explanatory, variables included social rank, time to conception, and age. Because we were specifically interested in a male's preference as it was expressed among multiple females, and because up to 12 females were present in a given session at the same time, we assigned each female in the session a value for rank, time to conception, and age, relative to the other females in the session. All three explanatory variables were simultaneously used in the model and were analyzed using Logit Estimate model in the Systat 8 statistical software package (Systat 1998).

Other statistical analyses

We presented mean values as $\tilde{X} \pm$ SE. We analyzed data that were not normally distributed using non-parametric tests. Two-way ANOVAS were conducted on rank-transformed data to test for interactions, since interaction analyses were not possible using the Kruskal-Wallis one-way analysis of variance. We considered differences between groups to be statistically significant when P<0.05. Where we conducted post-hoc tests, P values were calculated using a Bonferroni adjustment for multiple comparisons.

RESULTS

Rates of affiliative behaviors in relation to conception

We first examined rates at which males directed affiliative behavior toward females near the time of conception during the 2-year period starting in June 1997 and ending in June 1999. Here, we divided male affiliative behaviors into three categories: approaches, investigatory behaviors, and courtship behaviors, as described above in Methods. Data included affiliative interactions observed within 202 male-female pairs, involving 20 females and 21 immigrant males. Time relative to conception had a significant influence on male approach behaviors (Figure 3.1; Kruskal Wallis: approach: KW₆=21.918, P=0.001; investigate: KW₆=27.844, P<0.001; court: KW₆=11.083, P=0.086). Interestingly, the highest rates of approach and investigatory behaviors were observed in the

Figure 3.1. Mean daily rates (\pm SE) at which males engaged in (A) approaches, (B) investigatory behaviors, and (C) courtship behaviors, plotted as a function of time relative to conception in targeted females. Interval '0' represents the date of conception \pm 2 weeks. Remaining intervals represent month-long periods surrounding the date of conception. N=202 male-female pairs involving 20 females and 21 immigrant males observed together during each of 7 month-long intervals. Investigatory behaviors included sniffing, licking, and greeting. Courtship behaviors included approach-avoid behavior, presenting, bowing display, and pawing the ground. Note that all Y-axes are on the same scale.



Monthly interval surrounding conception

month directly preceding the female's fertile period. Although rates at which males emitted courtship behaviors were highest during the month of conception and the month preceding conception, the differences apparent in this analyses did not quite satisfy our criterion for statistical significance.

Rates of male affiliative behavior also varied as a function of female rank category and female age class (Figure 3.2). Female social rank alone did not significantly influence male affiliative behavior. However, we found a significant interaction between female rank category and female age class for male approaches, investigatory behaviors, and courtship behaviors (2-way ANOVA: approach: $F_{4,117}$ =4.250, P=0.003; investigate: $F_{4,117}$ =2.407, P=0.05; court: $F_{4,117}$ =3.346, P=0.012). Males tended to prefer older to younger females in the middle- and low-ranking categories.

Male hyenas did occasionally exhibit flehmen after sniffing female urine and sometimes after sniffing various parts of the female body (17 observations of immigrant males exhibiting flehmen out of 411 male investigatory behaviors in 1997-1999). In the past, male hyenas have only been observed exhibiting flehmen after sniffing female urine (Holekamp et al., unpublished data). In this study, we also observed male hyenas exhibiting flehmen after sniffing a female's hind legs, feet, and derriere.

Immigrant males rarely greeted with adult females. When they did, the male usually approached the female first, stood with his head towards her tail, and lifted his inside hind leg over her nose. The female then sometimes lifted her inside hind leg over the male's nose, and both members of the pair mutually

Figure 3.2. Daily rates at which males engaged in (A) approaches, (B) investigatory behaviors, and (C) courtship behaviors, plotted as a function of female social rank and age categories. Daily rates were rank transformed to perform 2-way ANOVA on data that were not normally distributed (see Methods for details). Transformed rates are plotted here (mean±SE).



sniffed each other's genitalia. During the 5-year period, 1991-1995, we observed only 6 symmetrical and 6 asymmetrical greetings between immigrant males and adult females. Both immigrant males and adult females initiated asymmetrical greetings to members of the opposite sex on three separate occasions, exposing their genitals to the other hyena to sniff, but the other member of the pair did not respond by lifting its own hind leg on these occasions. These 12 total greetings between immigrant males and adult females represented only 1.5% of all malefemale affiliative interactions observed during this 5-year period. All immigrant males (N=6 different males) that greeted with adult females (N=9 different females) were ranked either 1 (N=6 greetings), 2 (N=4 greetings), or 3 (N=2 greetings) in the male hierarchy. Ranks of females who greeted with males ranged from 1 to 26, including the highest and lowest ranking females present in the clan.

Adult natal males performed relatively few affiliative behaviors toward adult females. Ten different adult natal males approached, investigated, or courted 16 different females, representing only 4% of all affiliative interactions observed between adult males and females from 1997 to 1999. The youngest age at which natal males were first observed courting or mounting adult females was 19.7 months, although the average age of all natal males observed courting and mounting females was 36.4 ± 0.9 mos (N=20 adult natal males). We were interested to see whether female responses to male affiliative behavior varied with male status, so we next examined rejection coefficients for natal versus immigrant males. Rejection coefficients (RC) for immigrant males were

significantly higher than were those for natal males (Mann Whitney U: U_1 =3042.000, P=0.001), suggesting that females responded more aggressively to sexual interest expressed by immigrant males than to that exhibited by natal males.

Mating behavior

Between 1988 and 1999, only 39 observation sessions occurred during which observers saw male hyenas mounting females. These consisted of 20 'complete' copulations, which included intromission and ejaculation by immigrant males, and an additional 19 mounting events, with or without intromission, but not including ejaculation (Table 3.1). 'Mating' and 'mating sessions' thus both refer to the observation sessions that included both mounting with and without intromission. Seventeen immigrant males mounted or copulated with 18 adult females, and five adult natal males mounted four different adult females.

Mating behavior occurred throughout the year, and the monthly distribution of sessions in which mating was observed reflects the pattern of known conceptions recorded for the Talek clan, with both mating behavior and number of known conceptions peaking in August. Most (35/39) mating sessions occurred during our morning observation hours, between 0530 and 0930 (Figure 3.3A). Three more took place between 1000 and 1230, and one took place at 1850, during evening observation hours. Overall, mating sessions took place at significantly higher rates during the morning than afternoon observation hours (Figure 3.3B; Mann-Whitney U: U_1 =25.000,

TABLE 3.1. Sample sizes for mating sessions. In the text and in all analyses, mating sessions refer to sessions in which we observed mounting, including mounting both with and without intromission.

	# Sessions	# Males mount or mate / session	# Other individuals present / session
Mounting without clear intromission	19	1	0-8
Total sessions with mounting only	19		
Mounting with intromission (copulation)	18	1	0-8
Mounting with intromission (copulation)	2	2	0-1
Total sessions with copulation	20		
Total mating sessions (mounting with and without intromission)	39		



Figure 3.3. (A) Times of day at which matings (N=39) were observed in relation to total number of hours of observation. (B) Daily mating rate calculated for morning and evening hours of observation. AM represents 0500-0900 hours, and PM represents 1600-2000 hours.

P=0.007). It should be emphasized, however, that we rarely followed hyenas between the hours of 2000 and 0500 hours, so additional matings may have taken place during these nighttime hours.

Copulations generally involved a predictable sequence of events. The male first made a series of approaches towards the female, often rushing at her as if to mount, then veering off at the last second. After a few approaches, the male then mounted the female from behind, attempting repeatedly to achieve intromission. This task was apparently made extremely difficult by the female's elongated clitoris with a forward facing opening. The male had to squat down and under the female, so low that his rump was sometimes on the ground, to manipulate his erect penis into the female's flaccid phallus (Figure 3.4A). This typically took several attempts over a period of several minutes. Once intromission was achieved, the male then assumed the 'standard' position mounted behind the female, stretching the female's flexible phallus posteriorly (Figure 3.4B). Some thrusting usually occurred at this time. Finally, the male remained still (no thrusting) and remained mounted, sometimes licking the female's back but never biting her, and ultimately resting his chin and chest on the female's back for several minutes (Figure 3.4C). We were unable to determine whether or not a copulatory lock occurs at this time.

Figures 3.5 and 3.6 present examples of typical mating sequences. It can be seen in both of these figures that one to several short mounts preceded each long mount. Entire sequences of mounting, thrusting and resting, like those shown here, were

Figure 3.4. Mating positions. (A) The male squats behind the female as he attempts to achieve intromission. (B) The male stands and thrusts after achieving intromission. (C) The male rests his chin and chest on the female's back after ejaculation. The subadult offspring of the mating female can be seen standing behind his mother in (C).



в

с



Figure 3.5. Temporal patterning of mounting behavior for a single male (WALD) mounting a single female (SEIN) when no other hyenas were present. Observers remained with this pair through minute 50.



Figure 3.6. Temporal patterning of mounting sequences for two males (A=MER, B=EXC) alternately mounting a single female (GOL) during a single observation session. Observers remained with these animals through minute 150. MER was higher-ranking than EXC, and each male sired one of the cubs in the twin litter produced 110 days after these copulations were observed.

sometimes repeated several times over the course of several hours, and possibly longer, as we were unable to determine whether additional copulations took place prior to or following our observations of mating. However, we never observed any one mating pair copulating repeatedly on successive days. Figure 3.5 depicts a single male mounting a single female when no other hyperas were present. We observed this type of situation, when only the mating pair was present, on 15 occasions, 10 of which involved only one long mount, as represented in the figure. Figures 3.6A and B depict two males mating with one female during the same observation session. We observed two males alternate mating with the same female on two separate occasions, and observed two males mate with the same female in succession (one after the other but without repeated alternations) on a third occasion. In 24 of the 39 (62%) mating sessions, hyenas other than the focal pair were present (\overline{X} =2.6 ± 0.4 other hyenas present, range=1-8). These other animals included adult females, adult males, and subadults, both related and unrelated to the mating female. During three of these 24 sessions (12.5%), a second adult male attempted to interrupt the mating pair. Twice, the individual who interrupted was an immigrant male higher-ranking than the mating male, and the interrupting male succeeded in gaining access to the female on these occasions. In each of these two cases, the two immigrant males present alternated mounting the female (as in Figures 3.6A and B). In one of these sessions, the interrupting, dominant male was only very mildly aggressive to the subordinate male while the latter was mounted. An approach by the dominant male, accompanied by a mild threat, was usually

enough to persuade the subordinate male to dismount. However, the subordinate male was not deterred for long: as soon as the dominant male moved away, the subordinate male mounted the female again.

On only one occasion did we observe any severe aggression between two males competing over an estrous female. Here again, the subordinate male was not obviously deterred by the dominant male's approaches, and he persisted in mounting the female. As the session proceeded, the inter-male aggression escalated, with the dominant male repeatedly biting the subordinate male who was mounted, and eventually the two males had a standing fight. This fight represented the most intense aggression we ever observed between males competing over an estrous female. Finally, on a different occasion, an adult natal male interrupted a mating pair by approaching them, but the natal male made no attempt to mount the female after interrupting the mating of his rival.

We recorded a total of 462 mounts in the 39 mating sessions observed. Of those 462 mounts, 275 had an accurate recorded duration to ± 2 seconds, and mount duration ranged from 1-1080 seconds (Figure 3.7; \overline{X} =40.0 \pm 6.9 s). Mounts were clearly divisible into short mounts, ranging in duration from 1-45 s (\overline{X} =5.3 \pm 0.5 s), and long mounts, which lasted longer than 100 s (\overline{X} =302.9 \pm 32.2 s, range=120-1080 s). Long mounts were observed in 20 mating sessions. Males performed an average of 1.8 \pm 0.3 (range 1-5) long mounts during these 20 sessions. We calculated the number of short mounts performed during intervals between long mounts, as well as the duration of inter-mount intervals.



Figure 3.7. Duration of 275 mounts observed during 39 mating sessions, suggesting a possible functional distinction between short (<60 s) and long (>100 s) mounts.

Males performed an average of 1.9 ± 0.9 (range 0-11) short mounts during the inter-mount interval. It took males an average time of 24.0 ± 6.7 min (range 1-86) min) to achieve a second long mount. It is noteworthy that two of the three longest inter-mount intervals were likely influenced by the interference of another male who achieved a long mount of his own during that time. When we calculated inter-mount interval length with these two samples excluded, we found that it took males an average time of only 16.8 ± 4.9 min to achieve a second long mount. Calculation of latency to first mount and number of short mounts preceding first long mounts was problematic since we usually encountered mating pairs after mounting behavior had apparently already begun. However, it is worth noting that it took males at least 18.0 ± 3.7 minutes and 12.9 ± 2.6 short mounts to achieve their first long mount. This is a minimum estimate based on the time elapsed between the beginning of the observation session and the time at which the male achieved his first long mount. Although successful intromission was not consistently observable, it is likely that males achieved intromission during all long mounts, because some males were occasionally observed to intromit even during mounts lasting less than one minute. Males performed more than one long mount in almost half (8/18) of the mating sessions in which we observed any long mounts at all. This suggests that multiple long mounts over the course of several hours or days may be typical of this species.

Only five natal males were observed to mount females. These mounting sessions involved only short mounts and did not resemble the mounting sequences performed by immigrant males in mating contexts (as depicted in

Figures 3.5 and 3.6). That is, natal males mounted at most a total of seven times, if at all, during a mounting session, they never remained mounted for longer than 1 s, they never performed any thrusting behavior, and none of the natal males ever achieved intromission.

Female cycle length and fertility

Although exact duration of the estrous period is unknown in this species, we observed one female mating with three different males in a single 60-hour period. Therefore, we surmise that estrus lasts at least 60 hours in this species. Our efforts to estimate female cycle length in the field based on female behavior have been largely unsuccessful. However, we have had the good fortune of observing five females mate multiple times in the course of several months. The shortest interval between two observed copulations, when the first copulation was apparently not fertile, was 23 days. A second interval was 64 days. Both these intervals involve multiples of roughly 3 weeks, thus suggesting that the non-fertile estrous cycle in *Crocuta* lasts approximately 3 weeks, rather than the 2 weeks commonly cited for this species (Matthews 1939; Lindeque 1981). The youngest age at which female hyenas were observed copulating during the present study was 24.2 mos, and the youngest female known to conceive was 25.4 mos.

In total we observed 39 copulations during 11 years of continuous daily observation. This represents a surprisingly small number of mating observations considering that, during the same time period, we recorded 158 conceptions associated with births of litters eventually observed above ground (\overline{X} =14.4 known

conceptions / yr). Furthermore, of the 39 mating observations, only 15 (38.5%) were associated with later production of known litters, suggesting that females might often need to mate two to three times, over repeated estrous cycles, to achieve a fertile conception or successful pregnancy. Our scant observations of mating would thus further suggest that the 158 litters produced might have required over 400 copulations, of which we saw at most only ten percent!

The likelihood of conception was apparently not influenced by the details of male sexual behavior. That is, whether or not a litter was observed 80-140 days after mating could not be predicted based on the mean duration of mounts per mating session (Mann Whitney U: U₁=62.000, P=0.247), the number of short mounts per session (Mann Whitney U: U₁=114.000, P=0.81), the number of long mounts per session (Mann Whitney U: U₁=84.000, P=0.17), or whether or not at least one long mount was observed during the session (χ_1^2 =0.869, NS).

Initiation of intersexual interactions

In Chapter 2 (Szykman et al. 2001), we used focal animal data to evaluate the roles played by adults of each sex in initiating associations by calculating rates of approach and following behavior performed during non-mating sessions when both males and females were present together. In these non-mating contexts, we found that males approached and followed females at significantly higher rates than females approached or followed males (Szykman et al. 2001). Using the same calculations as those utilized there, we here contrasted male and female approach and following behavior in 'mating' contexts to those same behaviors

observed in 'other', non-mating contexts. 'Mating' contexts refer to sessions during which we observed mounting behavior. 'Other' contexts include all other sessions, including observations of hyenas present at dents, at carcass sites, and at locations other than dens and carcasses. There were significant main effects and significant interaction effects for context by gender for both approaches and following behavior (Figure 3.8; 2-way ANOVA: approach: F_{1.61}=17.744, P<0.001; follow: F_{1.61}=13.967, P<0.001). We therefore conducted post-hoc t-tests on each pair of samples, using the Bonferroni adjustment for multiple pair-wise comparisons. During mating sessions, males approached females at significantly higher rates than females approached males (t_{25} =-3.949, P=0.001), and males also approached females at higher rates than vice versa in other contexts (t_{36} =-6.220, P<0.001). Both males and females approached individuals of the opposite sex at significantly higher rates during mating sessions than in other contexts (male: t_{30} =5.312, P<0.001; female: t_{31} =5.267, P<0.001). Following behavior showed a dramatically different result. While males spent a greater proportion of focal animal time following females in non-mating contexts than vice versa (t_{36} =-5.879, P<0.001), females showed a tendency to spend a greater proportion of their focal animal time following males than vice versa in mating sessions. However, this tendency was not statistically significant $(t_{25}=1.780, P=0.087)$. Females were significantly more likely to follow males during mating sessions than in other contexts (t_{34} =4.863, P<0.001), whereas male following behavior did not vary significantly between contexts ($t_{27}=0.140$, P=0.890).



Figure 3.8. Mean (\pm SE) hourly rates at which individuals approached, and mean (\pm SE) percent of focal animal time during which individuals followed, members of the opposite sex in two different contexts. Open bars represent adult males and solid bars represent adult females. The 'mating' context consists of sessions during which we observed mounting behavior. The 'other' context includes all other sessions, involving observations of hyenas present at dens, at carcass sites, and at locations other than dens and carcasses. Numbers over bars represent number of individuals included in each sample. * P<0.001

Male-female associations

We next examined temporal patterns in the associations between males and females, relative to the time of conception. Time to conception was significantly associated with the number of hours males and females were observed together (Figure 3.9A; repeated measures ANOVA: N=20 females, $F_{6,114}$ =5.707, P<0.001). Males spent significanty more time with females during the month surrounding conception than during any preceding month except the one immediately preceding, or the one immediately following, conception (Tukey's post-hoc comparisons). Furthermore, more individual males were observed associating with females during the months surrounding conception than at other times (Figure 3.9B; repeated ANOVA: N=20 females, F_{6,114}=5.832, P<0.001). This was not due to females being observed for more hours during the month around conception, because females were actually observed more during early lactation than during periods when they were most likely to conceive (see Chapter 2).

We next analyzed consecutive observation sessions to examine how consistently males were found with specific females around and preceding the time of conception. We further inquired whether these patterns differed for sires and non-sires, as well as whether there was any evidence of mate-guarding by sires. Previous association data indicated that males known to have sired the offspring of individual females associated significantly more closely with their mates than did other males (Szykman et al. 2001). Furthermore, sires spent significantly more time alone with their future mates than did males failing to sire



Figure 3.9. (A) Total time immigrant males were observed with 20 females, and (B) number of immigrant males that were observed associating with same females, during the months surrounding each female's conception. Letters over bars represent means significantly different from one another at P<0.05.

offspring, particularly during the months immediately preceding conception (Szykman et al. 2001). Those analyses used association indices, representing the proportion of observation sessions in which individual males and females were seen together, to measure the strength of male-female associations. Here, we examined the temporal distribution of these associations more precisely.

Individual males varied in the extent to which they associated with females around the time of conception (Figure 3.10). One male in particular (the 5th ranked male in Figures 3.10A and B) was consistently observed with females during the 2 to 3 weeks preceding conception, and clearly succeeded in fathering cubs using this strategy. Other males, of both high and low rank (ranks 2 and 16, respectively) succeeded in siring offspring without exhibiting a clear pattern of association with their future mate (Figures 3.10C and D). Thus, male hyenas may sometimes appear to 'guard' their mates, but intensive mate-guarding is clearly not necessary in this species to ensure that a male will sire the cubs of a particular female.

We can sometimes utilize information from long-term male associations with an individual female to infer female receptivity. In the field, we have successfully predicted dates of birth for several litters by adding 110 days (the period of gestation) to the last day of a male-female consortship, like those depicted in Figures 3.10A and B. This is a particularly useful tool, given that the female hyena provides minimal behavioral signs of estrus, and that we so rarely observe copulation.

Figure 3.10. Association patterns between four females and the males who sired (open diamonds) their litters (N=3 males; open diamonds in (A) and (B) represent the same male), and the 17 males present concurrently who did not sire (solid diamonds) their litters. Estimated day of conception is indicated by the solid vertical line. Although the number of consecutive sessions during which each female was observed varied, the time scales in (A)-(D) are all standardized to include the three months preceding and the first month following conception.



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Factors influencing male choice behavior

We evaluated male choices for 210 male-female pairs involving 20 male and 27 female hyenas using logistic regression. Male hyenas clearly preferred some females over others, and decisions made by male hyenas to stay with or pursue a particular female were significantly influenced by certain female characteristics. Males who sniffed a particular female during an observation session were significantly more likely to stay with that female when she was closer to the time of conception, than to leave the scene or to stay with another female (Table 3.2A). Female social rank and age did not significantly influence males' choices to stay with females or leave them after sniffing them (Table 3.2A). Given a choice among several females, male hyenas exhibited the strongest preferences for, and actively pursued, high-ranking females, but males also preferred females when they were closer to the time of conception (Table 3.2B).

DISCUSSION

Male affiliative behavior and female attractivity

Female dominance and masculinized genitalia are among the many unique attributes of spotted hyenas that make them interesting subjects for the study of sexual behavior. Although some of the general affiliative and investigatory behaviors described above for hyenas also occur among other members of the order Carnivora (e.g. lions: Schaller 1972), many of the courtship behaviors discussed in this chapter are unique to this species. The approach-avoid
Table 3.2. Results of logistic regression examining effects of independent variables (female characteristics) on male choice behavior.

A Sniff-and-stay

Female characteristic	Estimate	S.E.	t-ratio	p-value
Rank	-0.064	0.077	-0.823	0.410
Time to conception	-0.395	0.085	-4.630	<0.001
Age	0.037	0.074	0.507	0.612

Log likelihood=-292.896 N=492 Correct predictions=62.0%

B Choice

Female characteristic	Estimate	S.E.	t-ratio	p-value
Rank	-0.222	0.087	-2.547	0.011
Time to conception	-0.162	0.08	-2.011	0.044
Age	0.007	0.078	0.093	0.926

Log likelihood=-303.077

N=459

Correct predictions=55.5%

behavior, bowing, and pawing the ground, all reflect some degree of motivational conflict and hesitancy on the part of the male, supporting the notion that male *Crocuta* fear females (Kruuk 1972; Holekamp and Smale 2000). It is likely that immigrant males have adopted these courtship behaviors as a consequence of their subordinate status, and that these courtship behaviors evolved from displacement activities motivated by conflicting male desires to approach the female and flee from her. The female

seems to take little notice of the male's amorous advances, and she does little to encourage him to pursue or approach her further.

Yet the male is not deterred. Despite the female's apparent disinterest, the male persists. Immigrant male hyenas approached, investigated and courted females throughout their reproductive cycle, but these behaviors peaked near the time of conception (Figure 3.1). From these data, we can infer that female attractivity increases prior to conception and declines thereafter. While males of all social ranks approached, investigated and courted females, only high-ranking males were observed to greet with them, confirming what East and colleagues (1993) and Mills (1990) found in their earlier observations of hyena greeting ceremonies. Rates of male affiliative behavior also varied as a function of female rank categories (Figure 3.2). Female age did not show any interaction with female rank among high-ranking females. This could be because age has little influence on female reproductive value in high-ranking females, who begin breeding at an early age, and cubs in their first litters often survive to

reproductive maturity.

Many male mammals exhibit flehmen. A male sniffs or licks some urine from a female, then he lifts his head with his lips parted, in a posture that may serve to transport odor to nasopalatine ducts and the vomeronasal system (Dagg and Taub 1970; Estes 1972; Doving and Trotier 1998). In this study, male hyenas were observed exhibiting flehmen after sniffing female urine, but also occasionally after sniffing a female's hind end. It has been reported that the vomeronasal organ functions to identify and interpret volatile components of female urine and vaginal secretions (Doving and Trotier 1998). Male hyenas are probably taking in female urinary pheromones when exhibiting flehmen after sniffing the female herself, despite no obvious presence of urine. This is likely because female hyenas are often observed to roll or lie down in their own urine, perhaps to cool off or cover themselves in their own tantalizing scent.

Natal male hyenas have been reported to court females at significantly lower rates than do immigrant males (Holekamp and Smale 1998). This is clearly not the result of females responding to natal males with higher levels of aggression. Rejection coefficients calculated in response to male affiliative behaviors indicate that females respond more aggressively to the advances of immigrant males than to those of natal males. This is an interesting paradox, as female hyenas behave most aggressively towards males with whom they will ultimately mate, and they virtually ignore advances by adult natal males, who rarely mate within their clan (Engh et al. 2002). Among domestic dogs (*Canis familiaris*), male behaviors and female responses are highly variable (Beach and

LeBoeuf 1967) as they are in *Crocuta*. Individual male dogs are acceptable to females, and individual females are discriminating among males, both to varying degrees. Beach and LeBoeuf (1967) ultimately found that rejection by the female did not affect the frequency of male-female interactions or the probability of a copulatory lock. Similarly, in hyenas, aggressive responses by the female did not appear to discourage immigrant males.

Mating behavior and female receptivity

Copulations are rarely observed in field studies of *Crocuta*. The small number of observations of copulation may be because *Crocuta* are mating at night, as suggested by Kruuk (1972) and Frank (1986b). It is also possible that mating pairs actively try to isolate themselves to avoid interference by other clan members. This would also make it more difficult for us to find and observe the mating pair. In fact, on several occasions during mating sessions, the courting pair vanished into thick bushes after the male had mounted the female once or twice, but had not yet achieved intromission. Thus, we were unable to continue to observe the pair.

Tentative approaches by the male, and attempted mounts that are aborted at the last second, may function to help the male build up the courage to mount the female, an idea suggested by S.E. Glickman (pers. comm.). Also, these approaches may serve to test the female's receptivity, as she may respond by turning to chase the male, or she may stand still ready to accept him. The female

standing still and lowering her head appears to be a clear signal to the male that it is safe to mount her.

The thrusting we occasionally observe prior to intromission may help the male find the female's vaginal opening and line the male up properly for successful intromission, as suggested for the same behavior observed in domestic dogs (Beach and LeBoeuf 1967) and cats (*Felis domesticus*: Beaver 1992). Repeated mounting without intromission is not commonly reported in other carnivores. Male hyenas appear to have greater difficulty achieving intromission than other male carnivores due to the strange genital morphology of the female hyena, with her long, flexible phallus and urogenital opening facing anteriorly. The extended time the male spends mounted, not thrusting, and resting his chest on the female's back, might represent a post-ejaculation period serving to facilitate sperm transfer, similar to the copulatory lock in canids (Asa and Valdespino 1998). Although we were unable to observe in the present study whether or not a copulatory lock occurred in *Crocuta*, prolonged intromission by the male hyena might serve to enhance fertility.

We observed male hyenas perform multiple ejaculations, as inferred from repeated long mounts in which the male rested his chin on the female's back, during a single mating session (e.g. Figure 3.6). This suggests that females may require multiple matings, both during a single estrous cycle and over successive estrous cycles, to ensure conception. Fewer than half of the matings we observed ultimately resulted in the production of a litter, and we have observed individual females each mating with the same male, and sometimes with a

different male, over two estrous periods within several weeks of each other. Female hyenas might require multiple matings within a given estrous period because they are induced ovulators. Presence of new corpora lutea in the ovaries of captive female spotted hyenas not recently exposed to males suggests that females can ovulate spontaneously, but that does not rule out the possibility that they are induced ovulators in the context of mating (N. Place and A. Conley, pers. comm.). In addition, there may be unusual obstacles to conception in female *Crocuta*, perhaps resulting from their masculinized morphology and/or physiology (Frank et al. 1995b; Frank 1997).

Maintenance of associations and female proceptivity

Male hyenas follow and attempt to maintain proximity to females, sometimes associating with certain individual females as closely as females do with their own offspring (Szykman et al. 2001). Furthermore, association patterns between adult males and females clearly have some relevance to sexual behavior in hyenas, as male hyenas who sire cubs associate more closely with their future mates than do males who do not sire cubs (Szykman et al. 2001). These associations in *Crocuta* are not usually cooperatively maintained by both partners as commonly occurs in primate consortships (Stern and Smith 1984; Small 1990), nor are they as exclusive. However, we found here that females approached and followed males at significantly higher rates during mating sessions than during observations conducted in other contexts (Figure 3.8). This suggests that female approaching and following behavior directed at immigrant

males should appropriately be considered to be proceptive behavior, indicating the female's active interest in mating. This subtle behavioral change, may be the only demonstration of proceptive behavior by the female spotted hyena. The subtlety of proceptive behavior in female hyenas stands in stark contrast with other carnivore species in which females commonly exhibit obvious proceptive behavior. For example, a lioness in estrus repeatedly approaches and rubs her chosen male, rolls and swishes her tail in front of him, and presents her genital area for him to sniff, frequently initiating mating (Schaller 1972). Domestic cats sometimes show dramatically exaggerated forms of these same behaviors. adding to them loud vocalizations to call for males (Beaver 1992), to which any owner of an intact female cat can attest. Proceptive behavior by female domestic dogs may appear more subtle than in cats, but it still is very clear; an estrous female seeks out a male and presents her anogenital region near the male's muzzle for his inspection (Beach et al. 1982). Early androgen exposure during early development in Crocuta (Licht et al. 1992) might possibly have an attenuating effect on female proceptive behavior in this species.

Males make choices

Sniffing females often provides male mammals with information about female reproductive condition via pheromones (reviewed in Bronson 1989). Male hyenas frequently sniff female conspecifics, presumably to evaluate each female's reproductive state. It is therefore very interesting that time to conception emerged here as the only significant female characteristic influencing a male hyena's

decision to stay with a female after he has sniffed her (Table 3.2A). A male's 'choice', indicated by his persistent following of, or extensive courtship behavior directed toward, a particular female, may reflect a decision-making process by the male after which he invests more time in that particular female. The male's choice behavior appears to be influenced by the female's time to conception, and may be affected even more strongly by female social rank (Table 3.2B). We interpret these data to indicate that male hyenas can discriminate among female hyenas, and that they may make strategic choices based on a female's social rank and her time to conception. Overall, the models tested in the logistic regression may not have very strong predictive value, but the significant effects of rank and time to conception variables suggest that these parameters do indeed influence male behavior. In Chapter 2, we similarly found that males prefer to associate with high-ranking females and females most likely to be fertile. It is currently unknown how males evaluate female rank in this species.

Temporal patterns in male-female associations suggest that a strategy of 'consorting' with a particular female may be more successful for some males than for others. Individual males may adopt one of several different reproductive strategies to maximize their own reproductive success (e.g. Figure 3.10). Our association data also suggest, in conjunction with our observations of two males alternating copulations with a single estrous female (Figure 3.6), that mate guarding is not a strategy commonly utilized by male *Crocuta*. Interactions among resident immigrant males are generally characterized by low levels of aggression, even when access to an estrous female is at stake (Kruuk 1972;

Frank 1986b). Here we observed only one incident of severe aggression among males competing over access to estrous females, and most male-male interactions at such times are surprisingly amicable.

In this study, we have presented several years of detailed observations from the field to further describe and elucidate spotted hyena sexual behavior. Courtship among hyenas is unusual among mammals, not merely because it reflects some degree of hesitancy on the part of the male, but because male hyenas experience such extreme conflicting desires to approach the female and flee from her. The locomotor patterns observed during copulation are unique due to the female's masculinized genitalia: the smaller, subordinate male hyena must insert his erect penis into the female's forward-facing phallus, a feat that may take several minutes to achieve. Finally, some male hyenas may use close associations, or 'consortships', to increase their chances of mating with a particular female. These data, in conjunction with the data presented in Chapter 2, suggest that individual male hyenas may adopt different reproductive strategies to attract and acquire mates.

Chapter 4

RARE MALE AGGRESSION DIRECTED TOWARD FEMALES IN A FEMALE-DOMINATED SOCIETY

INTRODUCTION

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Aggression directed by males toward conspecific females occurs in a wide range of animal taxa (e.g. insects: Stone 1995; birds: McKinney et al. 1983; mammals: Smuts and Smuts 1993). Male mammals have been observed to behave aggressively towards females in a variety of contexts. For example, male olive baboons (Papio cynocephalus) attack females during feeding competition, in defense of a close female associate or her offspring, and during disputes with other males (Smuts 1985). In addition, male aggression toward females often occurs in a sexual context (reviewed in Clutton-Brock and Parker 1995). Males in mammalian species ranging from feral horses (Equus caballus) to hamadryas baboons (*Papio hamadryas*) chase and bite females to keep females in their groups and to discourage females from associating with rival males (Stammback 1987; Linklater et al. 1999). In other baboons and some macaque species, males behave aggressively toward females to punish persistent refusal to mate (Smuts 1987a). In some species, males may physically force females to copulate with them, as observed in orangutans (*Pongo pygmaeus*: Mitani 1985). All of the species mentioned above exhibit strong sexual dimorphism. That is, males are larger and better armed than females, and males dominate females. Here we

document male aggression against females in the spotted hyena, a species in which females exceed males in body size by approximately 10%, and in which females are socially dominant to males (Kruuk 1972).

In addition to discerning that female *Crocuta* are socially dominant to males, Kruuk (1972) observed that the only circumstances in which adult male hyenas behave aggressively toward adult females involve what he called "baiting" behavior. During baiting, the female hyena sits or lies in a crouched position with her ears flattened back against her head and her teeth bared, while multiple males surround her with their ears cocked forward and their elevated tails bristled. The males often rush at the female, and attempt to sniff or bite her, and she sometimes sustains serious wounds from their attacks (Kruuk 1972). The female defends herself, often lunging at one or more of the participating males, and she may inflict serious wounds on them. Kruuk (1972) was puzzled by the adaptive significance of this odd behavior pattern, but he doubted that baiting was related to sexual behavior, during which males show fear and behave extremely submissively to females.

Here we present data suggesting that this rare male aggression directed toward females is tightly coupled to female reproductive state in spotted hyenas. First we compare rates at which intrasexual and intersexual aggression occur among free-living *Crocuta*. We then examine the circumstances under which male hyenas behave aggressively toward females, and consider the possible proximate causes and adaptive function of male aggression directed toward females in this species.

METHODS

Subject animals and study area

Spotted hyenas are gregarious carnivores that live in social groups called clans. Each clan contains one to several matrilines of adult females and their offspring, as well as one to several immigrant adult males. Clans are rigidly structured by hierarchical rank relationships (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986b; Holekamp and Smale 1990; Mills 1990; Holekamp and Smale 1993), and an individual's social rank determines its priority of access to food and other resources (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986b). Adult females are socially dominant to all adult males not born in the clan (Kruuk 1972; Smale et al. 1993). Female hyenas are philopatric, but all males disperse to new clans sometime after reaching reproductive maturity at 2 years of age (Smale et al. 1997). Although natal males may remain in their natal clans up to 51 months after puberty (Smale et al. 1997; Holekamp et al., unpublished data), and although they occasionally participate with immigrant males in coalitionary aggression directed at females, such participation is rare (Holekamp and Smale 1998). We therefore focus exclusively here on aggressive interactions between immigrant males and adult females.

We conducted this study in the Talek area of the Masai Mara National Reserve, in southwest Kenya. This is an area of open, rolling grasslands grazed year round by large concentrations of several different ungulate species. The

subject population was one large *Crocuta* clan inhabiting a home range of approximately 65 km² (Frank 1986a; Boydston et al. 2001). We identified all hyenas in the Talek study clan individually by their unique spot patterns, and we determined their sex from the dimorphic glans morphology of the erect phallus (Frank et al. 1990). We established mother-offspring relations on the basis of regular nursing associations. We photographed immigrant males immediately upon their first sighting in the Talek area. We calculated the duration of a male's tenure in the study clan at the time when he performed a behavior of interest by subtracting his date of arrival from the date on which that behavior was observed. In this study, we monitored Talek hyenas continuously from June 1988 through August 1999.

Behavioral observations

Throughout the study period, we recorded all approaches, appeasements, and aggressive behaviors among hyenas as critical incidents ("all-occurrence sampling" of Altmann 1974). We recorded an approach when one individual moved to within 1 m of another individual. Appeasement behaviors included head bob, carpal crawl, giggle, squeal, back off, and assumption of the body posture in which the ears are flattened back against the head, the tail is tucked between the legs, the body is lowered and bent, and the head is down. Listed in order of increasing intensity, aggressive behaviors included displace, intention movement to bite, push, stand over, lunge, rush, snap, chase, and bite (Holekamp and Smale 1990). Any interaction between two or more hyenas in which we observed

one or more of these individual acts of aggression was called an aggressive interaction, or attack. For some analyses we divided acts of aggression into "low intensity" and "high intensity" categories. The former included displace, intention movement to bite, push, stand over, and lunge. Individual acts of aggression considered to be of high intensity included rush, snap, chase, and bite. If an aggressive interaction involved only one aggressor and one recipient, we called it a dyadic interaction. By contrast, a coalitionary interaction involved two or more aggressors attacking a single target animal.

We assigned each aggressive interaction a location, the physical locality where the interaction took place, and a context, the social situation immediately preceding the attack. Locations included "dens," "kills," and "other" places away from dens or food. "Dens" included both natal and communal dens, and "kills" included both freshly killed ungulates and old ungulate carcasses. We observed aggressive interactions among adult hyenas in the following five contexts: during competition over food, in defense of cubs, when one hyena was being "pesky" (e.g. persistent sniffing, etc.) to another, during displacement aggression ("scapegoating"), and in situations where none of these other possibilities applied, such that the aggression appeared to be entirely unprovoked.

We calculated daily rates at which adult hyenas of each sex emitted and received aggression for a portion of the study period, between June 1988 and May 1995. For this analysis, we divided the number of aggressive interactions involving each individual as either attacker or target by the total number of hours each individual was observed during that period, and then multiplied this number

by 24. In a separate analysis, we calculated the percent of attacks emitted and received by adults of each sex that occurred in coalitions by summing the number of attacks occurring in coalition, dividing by the total number of attacks, then multiplying this by 100.

We determined intrasexual social ranks of individuals in the clan's dominance hierarchy from outcomes of several thousand dyadic agonistic interactions during which one individual exhibited appeasement to the other, whether or not the appeasement was elicited by aggression from the social partner, as described by Holekamp & Smale (1990) and Smale et al. (1993). By convention, the highest-ranking (alpha) individual was assigned a rank position of one. To evaluate frequency of male aggression towards other males as a function of social rank category, we calculated rates of male attacks on other males during an 8.6-month period for which the male hierarchy was completely stable. When contrasting rates of aggressive behavior by males directed at both other males and females, we divided male social ranks into three equal rank categories, high, middle, and low.

For each aggressive interaction in which one or more males attacked a female, we recorded the identity and social rank of the female target, and the number of males participating in each attack, as well as their identities and social ranks. For each attack, we also recorded, whenever possible, the particular acts of aggressive behaviors emitted by each individual male, the female's responses to each individual male (classified simply as aggressive or non-aggressive), and the location, context, and duration (in minutes) of each attack. For analyses

examining baiting rates and individual acts of aggression exhibited by different individual males toward females, we used a subset of the males (N=32) for which critical incident data on individual acts of aggression were recorded.

We first determined whether rate or intensity of male aggression varied with female reproductive condition. To do this, for the date on which each intrasexual aggressive interaction was observed, we assigned each female to one of the six reproductive states described below. If we were uncertain about a female's reproductive condition when she was the target of male aggression, then that interaction was excluded from relevant analyses. We defined the six reproductive states as follows.

Nulliparous (N): Here females were older than 24 months, but had not yet conceived their first litter. The youngest age at which female *Crocuta* in this population first conceived was 25.4 months, and females were observed to copulate as early as 24.2 months of age (see Chapter 3). Age at first parturition can readily be established in this species because the female bears an enlarged clitoris (the pseudopenis) which tears when her first litter is born (Frank and Glickman 1994), and heals leaving a large patch of pink scar tissue on its posterior surface. Here the condition of this surface was carefully monitored in all nulliparous females older than 2 years, during the phallic erections so commonly exhibited when hyenas greet each other (Kruuk 1972; East et al. 1993). Thus, even when a female's first litter was lost before her cubs first appeared above ground, it was possible to reliably determine when (to within 10 days) she had delivered her first litter.

Pregnant (P): The duration of pregnancy in *Crocuta* is 110 ± 1.0 days (Schneider 1926; Kruuk 1972; M.L. Weldele, pers. comm.). We assigned birth dates to litters by estimating cub ages when we first observed cubs above ground at natal or communal dens. Cub ages could be estimated to \pm 7 days based on their pelage, size, and other aspects of their appearance and behavior. We calculated conception dates by subtracting 110 days from estimated birth dates, thus resulting in conception dates that were accurate to \pm 7 days. Although most female hyenas wean one litter weeks or months before conceiving another, females of all social ranks have occasionally conceived while still nursing a previous litter (Holekamp et al. 1996). Females known to be pregnant while still lactating were assigned a reproductive state of 'P' (see above).

Lactating (L1, L2, L3): The lactation interval began with the birth of a litter and ended with its weaning. Weaning conflicts and cessation of nursing indicated when cubs were weaned. Weaning conflicts between cubs and their mothers are easily observed in this species due to the characteristic begging behavior and loud whining exhibited by cubs at this time. We recorded all weaning conflicts in field notes as critical incidents (Altmann 1974). In determining weaning dates, we searched all field notes for observations of nursing behavior when mother and cub were found together. If mother and cub were not found together frequently after the last observed nursing bout, we identified the weaning date as being midway between the last nursing bout and the next sighting of mother and cub together without nursing. However, we only used intervals of 20 days or less in this analysis, so all weaning dates used here were accurate to within ± 10 days.

Lactation periods vary in length among Talek females from 7 to 21 months (Holekamp et al. 1996; Holekamp and Smale 2000). Therefore, to compare rates and intensity of male aggression among females with different lactation period lengths, we divided the total period of lactation into three periods of equal length (represented as L1, L2, and L3) for each female.

Other (O): Females assigned to this condition had weaned one litter but had not yet conceived their next litter. Little is known about the reproductive cycle of the spotted hyena. Matthews (1939) and Lindeque (1981) suggested that estrous cycles in this species recur every 14 days, but there are no apparent morphological indicators of estrus in female spotted hyenas as there are, for example, in many cercopithecine primates (reviewed in Melnick and Pearl 1987), and copulations are rarely observed in *Crocuta*.

For each female in each reproductive state, we calculated a daily rate of male aggression toward that female using the formula: (number of male attacks directed at each female per reproductive state/total number of hours the female was observed in that state * 24 hrs). We assigned any adult female who was observed but not attacked by males while she was in a given reproductive state a daily rate of zero for that state.

We next examined the occurrence of male aggression relative to the date of a female's nearest conception. We calculated time relative to conception by subtracting the female's nearest conception date from the date on which she was observed being attacked by males, and then dividing occurrence of these attacks into month-long intervals. Together, the two 2-week intervals on either side of the

conception date represented month "zero".

Paternity determination and statistical analysis

We used determination of paternity to compare rates of male aggressive behavior toward each female *Crocuta* between males who sired her cubs and males who did not sire her cubs but who were present with that female during the period in which her litter was conceived. We used nuclear genetic markers to determine paternity of cubs born in the Talek clan (Engh et al. 2002). Between 1990 and 1999, we collected blood from 191 Talek hyenas anaesthetized with Telazol (2.5 mg/kg) delivered from a CO₂-powered rifle in a lightweight plastic syringe. We then immediately extracted DNA from whole blood using Puregene kits (Gentra Systems, Inc.), and stored the extracted DNA in liquid nitrogen.

We considered all adult males present in the clan at the time of conception as potential sires. Paternity assignments were based on 12 microsatellite markers described elsewhere (Libants et al. 2000). Briefly, we employed a maximum-likelihood approach (Thompson 1975; Meagher 1986) to assign paternity to cubs with known, genotyped mothers using the program CERVUS (Marshall et al. 1998). A male was considered the father of a cub when CERVUS assigned him as such with 95% confidence. A more comprehensive analysis of paternity in the Talek clan is presented by Engh et al. (2002).

Our behavioral data were not normally distributed, so we conducted all analyses using non-parametric tests. Mean values were presented as $\overline{X} \pm SE$, and we considered differences between groups to be statistically significant when

P<0.05. All statistical tests were two-tailed. Data collection periods varied for different analyses presented in the text, so please refer to Table 4.1 for details.

RESULTS

Sex differences in aggression

Adult male hyenas behaved aggressively toward other adults at far lower mean daily rates than did adult females (Figure 4.1; Mann-Whitney U: U_1 =409.000, P<0.001). Whereas males and females directed attacks at adult males at marginally different rates (Mann-Whitney U: $U_1=305.500$, P=0.07), the aggressive behavior of males and females toward adult females differed dramatically (Mann-Whitney U: U₁=444.000, P<0.001). Specifically, adult females were frequently aggressive toward other females, and although females tended to attack other females at higher rates than they attacked males, this difference was not statistically significant (Wilcoxon signed-ranks test: Z=1.855, N=23, P=0.07). In contrast to females, adult males very rarely attacked adult females, and did so at significantly lower rates than they attacked other males (Wilcoxon signed-ranks test: Z=-3.621, N=20, P<0.001). In fact, during over 30,000 observer hours watching Talek hyenas between 1988 and 1995, we observed only 256 individual acts of aggression in 195 attacks directed at adult Talek females by adult immigrant males compared to 1207 aggressive acts in 826 attacks on adult immigrant males by adult females during the same period. The 256 acts of aggression directed by males toward females involved 30 different adult females, and 40 different adult immigrant males participated in at least one of these

Table 4.1 Data collection periods for analyses presented in Chapter 4.

Analysis	Location	Data collection period		
All adult aggression	Figures 1, 2, 3	1988-1995		
Approaches and responses	Figure 4	1988-1999		
Baiting, general	Figures 5, 6, 7, 8, 9	1988-1999		
Male-male aggression	Text p. 124	1992-1993		
Baiting, sires vs. non-sires	Text p. 130	1991-1999		

Daily aggression rate



Figure 4.1. Daily rates (mean \pm SE) at which adult hyenas (N=23 females and 20 immigrant males) directed aggression toward other adults of each sex, controlled for the number of hours observed during which each adult was found in the presence of other adults of each sex between 1988 and 1995.

events. On average, each of these 40 males participated in 12.4 ± 1.8 (range: 1-51) attacks directed at adult females during his tenure in the Talek clan.

Intensity of aggression varied among intrasexual and intersexual attacks (Figure 4.2). Male attackers exhibited a significantly greater proportion of lowintensity aggression than we observed for female attackers, regardless of the sex of the target animal (χ^2_1 =94.52, P<0.001). Immigrant males exhibited the lowest proportion of high intensity aggressive behaviors when attacking adult females (χ^2_1 =26.02, P<0.001). Adult females exhibited the greatest proportion of high intensity aggressive behaviors when they attacked other females, though this proportion did not differ significantly from that of female attacks on males (χ^2_1 =1.73, NS). That is, 60% of individual acts of aggression by females were of high-intensity, regardless of the sex of the target animal.

In contrast to male-male aggression and aggression directed against either sex by adult females, most (76%) male attacks upon females occurred in coalitions (Figure 4.3). The proportion of total attacks on adult males that occurred in coalitions was similar for male and female aggressors (χ^2_1 =1.475, NS), but a significantly larger proportion of attacks on adult females occurred in coalitions formed by male aggressors than by female aggressors (χ^2_1 =232.99, P<0.001). Both males and females were more likely to form coalitions to attack females than to attack males (male attackers: χ^2_1 =161.2, P<0.001; female attackers: χ^2_1 =34.18, P<0.001).

Interestingly, females were significantly more likely to respond aggressively to males approaching them in an aggressive manner than when



Figure 4.2. Percent of total individual acts of aggression of high intensity that were directed by male and female hyenas against members of the same and opposite sex. Sample sizes indicate total number of individual aggressive acts observed between 1988 and 1995.



Figure 4.3. Percent of total attacks on other adults that occurred in coalitions. Sample sizes indicate total number of attacks observed between 1998 and 1995.

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males approached females without exhibiting aggression (Figure 4.4; χ^2_1 =44.05, P<0.001). In general, females responded aggressively towards males who approached aggressively, whereas they tended to ignore or investigate males who approached in a non-aggressive manner. Male hyenas thus increased their own risk of injury by behaving aggressively towards females.

Male aggression toward females

The number of males participating concurrently in attacks on adult females varied from one to six (Figure 4.5), with a mean of 2.5 ± 0.1 males joining forces per attack. On average, each male participating in these attacks directed 1.9 ± 0.1 (range: 1-19) individual acts of aggression at the female per attack. The two most intense forms of aggression, chasing and biting, were directed at the female in 15.6% of attacks. On average, male attacks on females lasted 3.84 ± 0.49 min (range 0.5 to 49 min), and attack duration did not vary with the number of males participating (Kruskal-Wallis: KW₅=3.204, NS).

Male aggression toward females was remarkably consistent in appearance, despite variability in the number of participating males. As males approached, they typically bristled their manes and tails, and surrounded and rushed the female, who crouched defensively in their midst with her teeth bared (Figure 4.6). Because the form of male attacks upon females was so consistent in appearance, and because it invariably resembled the intersexual aggressive interactions Kruuk (1972) called "baiting behavior", we adopted Kruuk's terminology. Thus "baiting" will henceforth be used here to refer to an



Figure 4.4. Female responses to male approaches. Sample sizes indicate numbers of male approaches observed for which female response was recorded.



Number of males participating

Figure 4.5. Numbers of individual males participating in 227 attacks on adult females. Sample sizes indicate numbers of interactions observed between 1988 and 1999 in which adult immigrant males attacked adult Talek females.



Figure 4.6. Example of male coalitionary aggression toward a female. Three males attack a female, who crouches in the center of this photograph with her teeth bared. Note the males' bristled tails, indicating a high level of excitement.



unprovoked aggressive attack directed by one to several adult males toward a single adult female, who responds by defending herself, and sometimes also with counter-aggression.

During most intrasexual aggressive interactions among natal Talek hyenas, and aggressive interactions in which females attacked immigrant males. approach or attack by one or more dominant animals elicited unambiguous submissive behavior from the targeted lower-ranking individual (Smale et al. 1993). That is, when aggressors approached targets, the aggressors' heads were held high, their ears were cocked forward, their manes and tails were bristled, and they stood their ground at the end of the interaction. During these ordinary aggressive interactions, subordinates flattened their ears back against their heads, tucked their tails between their legs, exhibited a facial expression called a "fear grin", bobbed their heads up and down, and backed off or fled to terminate the interaction. By contrast, males engaging in baiting behavior held their heads high with their ears cocked forward and manes and tails bristled, yet they often simultaneously bobbed their heads up and down, backed away spontaneously from the crouching female, and fled when singled out and chased by the targeted female. Thus, males participating in baiting appeared to experience conflicting desires to attack and flee. Although females targeted during baiting crouched to defend vulnerable body parts and flattened their ears back, they did not otherwise exhibit typical submissive behavior. The baring of teeth by baited females clearly involved the contraction of a different set of facial muscles from those contracted during normal "fear grins". When baited by a lone

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male, an individual female was readily able to demonstrate her dominant status, and she never backed off from, or submitted to, the male. However, when surrounded by a group of males, the female appeared to experience greater difficulty protecting herself from simultaneous attack by multiple males. Thus, males acting in groups launched attacks against dominant females, yet these coalitionary attacks did not result in any change in dyadic rank relationships between individual males and females.

Males baited females at a variety of locations where many hyenas were found together. Eleven percent of male attacks upon females occurred at dens, 26% occurred at sites of ungulate kills, and 63% occurred at locations away from dens or food (Table 4.2A). Whereas the duration of male attacks on females did not vary with location (Kruskal-Wallis test: KW_2 =3.269, NS), the number of participating males was significantly greater at kill scenes than at dens or other locations (Kruskal-Wallis test: KW_2 =13.841, P<0.001). This may be due to the fact that the average number of hyenas present at kill scenes is greater than the average number of hyenas found at dens or locations away from dens or food (Kruskal-Wallis test: KW_2 =535.225, P<0.001). Nevertheless, even when males outnumbered females at a kill scene, males were never able to usurp food from feeding females. At 886 fresh ungulate carcasses, at which both immigrant males and adult females were present during 640 hours of observation, not once was an adult immigrant male ever observed displacing an adult female from food.

Regardless of the location of these aggressive interactions, all male baiting of females appeared to be entirely unprovoked (Table 4.2B). No male

Table 4.2. Locations (A) and contexts (B) in which individual acts of aggressive behavior were observed between 1988 and 1995. (Note: aggression by males toward females observed between 1996 and 1999 are considered in the text but are not included in last column of this table.)

		Female-Fe	nale Female-Male		Male-Male		Male-Female		
Α	Location	Number	%	Number	%	Number	%	Number	%
	Dens	483	58.5	378	33.3	47	23.7	28	10.8
	Kills	307	37.2	391	34.4	102	51.0	67	26.2
	Other	36	4.3	367	32.3	51	25.4	161	63.1
	Total	826		1136		200		256	

в	Context	Number	%	Number	%	Number	%	Number	%
	Food	342	54.0	194	43.7	97	60.6	0	0.0
	Intervention	16	2.5	1	0.2	0	0.0	0	0.0
	Pesky	40	6.3	11	2.5	0	0.0	0	0.0
	Scapegoat	42	6.6	19	4.3	25	15.6	0	0.0
	Unprovoked	151	23.9	215	48.4	35	21.9	256	100.0

aggression toward females occurred during feeding competition, even when food was present at the location of the interaction. Furthermore, no baitings occurred during defensive interactions on behalf of another hyena, during displacement aggression ("scapegoat" in Table 4.2B), or in response to the female behaving in a "pesky" fashion toward males. By contrast, male-male aggression occurred in a variety of different contexts (Table 4.2B), most often during displacement aggression and feeding competition, where high-ranking males were able to displace lower-ranking males from desired resources.

Effects of male rank and tenure in the clan

Baiting rates varied significantly as a function of male rank category. High- and middle-ranking males baited females at higher rates than did lower-ranking males (Figure 4.7; Kruskal-Wallis test: KW_2 =28.683, P<0.001). This was not merely a function of high-ranking males behaving more aggressively in general. High-ranking males were no more aggressive toward other immigrants than were lower-ranking males, even when taking into account the number of potential targets available to males at each rank (Kruskal-Wallis test: KW_2 =0.202, NS).

Individual males baited females at rates that varied greatly (range: 0.408-6.360 baitings/day observed/tenure year in clan). There was a 15-fold difference between the minimum and maximum rates at which males baited females over their full tenure in the clan. However, due to the high variation among years for individual males, differences in baiting rates among individual males were not statistically significant (Kruskal-Wallis test: $KW_{31}=40.024$; NS). Nevertheless,



Figure 4.7. Daily rates (mean \pm SE) at which adult males in each of three rank categories baited females. Rank categories high, middle, and low represent male ranks 1-6, 7-12, and \geq 13, representing N=23, 18, and 8 males, respectively. Bars sharing same letters represent means not significantly different from one another.
individual males varied significantly in the number of individual aggressive acts directed towards females during each baiting (Kruskal-Wallis test: KW_{31} =65.610, P<0.001).

Male tenure is a better predictor of male reproductive success than is social rank (Engh et al. 2002), but here tenure could not predict baiting rates better than could male social rank. Daily baiting rates did not vary significantly with the year of male tenure (Kruskal-Wallis test: KW_{10} =15.384; NS). However, the proportion of males who were observed baiting females did generally increase with tenure in the clan (Figure 4.8; χ^2_{10} =20.796, P=0.02). Only 30% of males in their first year in the clan baited Talek females, whereas 50-70% of males remaining in the clan for 2 to 9 years were observed baiting females. All males remaining in the clan for 10 and 11 years were observed to bait females during their residence in the clan, although only two males persisted in the Talek clan for such a long period of time.

Effects of female rank and reproductive state

We observed females of all social ranks being baited. We found no significant relationship between daily baiting rates and female rank for females in any particular reproductive state, or overall (Spearman rank correlation: r_s =-0.301, N=18, NS). Furthermore we found no significant relationship between a female's social rank and either the number of males participating in her baitings (Kruskal-Wallis test: KW₁₈=20.311, NS) or the duration of her baitings (Kruskal-Wallis test: KW₁₅=18.807, NS).



Figure 4.8. Proportion of males observed baiting females per year of male tenure in the Talek clan. Numbers over bars represent the number of males represented in each year of tenure.

We found females being baited in all six reproductive states. We observed individual females in each reproductive state for 0-844.6 hours: \overline{X}_{N} =17.9 ± 8.4. \overline{X}_{P} =66.2 ± 14.8. \overline{X}_{1} = 120.5 ± 22.1. \overline{X}_{1} = 84.8 ± 18.2. \overline{X}_{1} = 56.0 ± 12.4. \overline{X}_{O} = 24.5 ± 6.8 hours per female. Neither the number of male participants per baiting (Kruskal-Wallis test: KW₅=6.557, NS), nor baiting duration (Kruskal-Wallis test: KW₁₅=8.544, NS) varied with female reproductive state. Proportions of high and low-intensity acts of aggression directed at the female by participating males did not vary with female reproductive state (χ^2_5 =3.77, NS). The proportion of baitings in which the target female responded to male aggression with an aggressive counterattack also did not vary with female reproductive state (χ^{2}_{5} =3.90, NS). Furthermore, in 68% of male attacks upon females that were either in the last trimester of lactation or between weaning one litter and conceiving the next, the female left her defensive crouch to threaten or chase one or more of the participating males, whereas in 32% of these interactions her behavior was entirely defensive. Thus, even when females were most likely to be sexually receptive, they usually responded to male aggression with aggressive counterattack.

Despite finding no significant differences among various details of baiting events and female reproductive state, female reproductive state did significantly influence the overall daily rate at which females were baited (Figure 4.9; Kruskal-Wall*i*s test: KW_5 =19.313, P=0.002). Baiting rates were significantly higher when females were in either the last third of the lactation interval, or between weaning



Female reproductive state

Figure 4.9. Daily baiting rates (mean \pm SE) per female for 22 females in various reproductive conditions observed between 1988 and 1995. "N" indicates nulliparous, "P" indicates pregnant, "L1" represents the first trimester of the lactation interval, "L2" indicates the second trimester of the lactation interval, "L3" indicates the third trimester of the lactation interval, and "O" indicates the period between weaning one litter and conceiving the next. Each female was observed in all six reproductive states.

and conception, than when females were in any other reproductive state. This is noteworthy because it is while females are in these two particular reproductive states that they are known to conceive. As mentioned earlier, females are probably often cycling during the "other" state, and must also often cycle during their last trimester of lactation, as females are sometimes known to maintain lactation and pregnancy concurrently. Mean baiting rates did not differ significantly between the L3 and O intervals (Mann-Whitney U test: U₁=300.000; NS).

Most baitings occurred during the last months preceding conception, with the greatest number of baitings observed during the month in which conception occurred (Figure 4.10). However, we never observed males mounting females during, or immediately after, a baiting event. The number of observed baitings dropped off sharply during the second month post-partum. Overall, males baited females at significantly higher rates before than after conception (Wilcoxon signed-ranks test: Z=2.184, N=97, P=0.029).

Baiting behavior compared for sires and non-sires

We next contrasted baiting behavior of males who sired cubs with that of males present concurrently with sires who failed to sire offspring. During the period 1991-1999 for which we have paternity data, we observed 201 baitings. Only 49 (14.4 %) of these baitings were observed within 6 mos of conceptions of litters with known paternity. When we compared the proportion of baitings in which sires and non-sires participated during 6-month time blocks before and after



Figure 4.10. Observations of baitings relative to the date of the nearest female conception. The interval labeled "0" represents the period starting 2 weeks prior to conception and ending 2 weeks after conception. The interval labeled "1" thus represents the period 2-6 weeks after conception, and so forth. For this analysis, we only included baitings that took place within one year of a female's nearest conception. (N=172 baitings of 27 females)

conception, we found no significant difference between sires and non-sires $(\chi^2_1=0.178, NS)$. Nor did overall rates of baiting behavior differ between sires and non-sires during this year-long time period (Mann-Whitney U test: U_1 =613.000, NS). However, while sires baited females at similar rates both before and after conception (Wilcoxon signed-ranks test: Z=0.356, N=11, NS), non-sires baited females at significantly greater rates before than after conception (Wilcoxon signed-ranks test: Z=2.106, N=86, P=0.035). We found no significant difference between the mean number of individual acts of aggressive per male per baiting for males who sired cubs during a female's reproductive cycle and those males who did not sire cubs of that particular female at that time (Mann-Whitney U test: U_1 =1096.000, NS). Finally, females did not appear to respond differently to attacks carried out by males who went on to sire cubs with them and those males who did not sire cubs. That is, the proportion of male attacks to which females responded aggressively did not differ between males who sired cubs and those who did not sire cubs during that female's reproductive cycle (χ^2_1 =3.342, P=0.07, NS).

DISCUSSION

Features of male aggression toward females

Kruuk (1972) first observed that female spotted hyenas appear to be more aggressive than males, and our results quantitatively confirm that females are indeed more aggressive than males in this species. Adult immigrant male spotted

hyenas exhibited intrasexual aggressive behavior at far lower daily rates, and at lower intensities, than did adult females, and rates of intrasexual aggression were higher for both sexes than were rates of intersexual aggression. Rates of male aggression toward females are far lower in Crocuta than in most other species for which male aggression directed toward females has been quantified (savanna baboons: Papio cynocephalus: Hausfater 1975: wild horses, Equus sp.: Berger 1983, Berger, 1986 #338; rhesus macaques, Macaca mulatta: Lindburg 1983; orangutans: Mitani 1985; e.g. chimpanzees, Pan troglodytes: Goodall 1986; Bercovitch et al. 1987; elephant seals: LeBoeuf and Mesnick 1990; Mesnick and Le Boeuf 1991; Connor et al. 1992a; dolphins, *Tursiops* sp.: Connor et al. 1992b; Japanese macaques, Macaca fuscata: Soltis et al. 1997). Darwin (1871) noted that males of most polygynous species are "more pugnacious" than females, but the reverse is clearly true in the spotted hyena. Male attacks on females occur rarely in hyenas and usually involve coalitions of several males. These attacks occur wherever male hyenas encounter adult females, except in the presence of lions or alien hyenas. In the present study, all male attacks on females were entirely unprovoked.

High- and middle-ranking males baited females at higher rates than did lower-ranking males, and the proportion of males who participated in baitings increased with immigrant male tenure in the Talek clan. Although reproductive success among male hyenas tends to increase with rank and tenure (Engh et al. 2002), we could detect no differences in rates or intensities of baiting between males who sired cubs and males who did not sire cubs. However, our failure to

detect such differences may be due to small sample sizes.

Females of all social ranks were baited with equal frequency and comparable intensity. Females were baited throughout the reproductive cycle. but baiting rates were highest during periods when they were most likely to conceive litters. Kruuk (1972) believed that baiting in spotted hyenas was unrelated to sexual behavior because, whereas high levels of intersexual aggression are observed during baiting, aggression by males toward females is never observed in association with copulation. Our observations confirm that male hyenas never attempt to mount females during baiting as, for example, do male dolphins during coalitionary "herding" of females (Connor et al. 1992b). However, our analysis of baiting behavior revealed a strong association with female reproductive state. Baiting rates here were extremely low during pregnancy and during the first two thirds of the lactation interval. Indeed, daily baiting rates were highest for females in the last third of lactation and during the "other" state, stages of their reproductive cycle during which they were most likely to conceive litters. In addition, the greatest number of baitings was observed during the month in which conception was known to occur. Similarly, male aggression toward females in many other mammalian species is most commonly observed near the time of conception (reviewed in Smuts and Smuts 1993). In fact, Smuts & Smuts further reported that they encountered very few instances of male aggression toward females in non-sexual contexts in their survey of the literature on non-primate mammals (see Table 1 in Smuts and Smuts 1993). Our data lead us to conclude that, despite its unlikely appearance,

male attacks on females are somehow related to sex in the spotted hyena.

Mechanisms and functions of male aggression toward females

Aggressive interactions involving multiple males were often preceded by social sniffing (Kruuk 1972) in which individual males bristled their manes and tails excitedly, leaned against each other, and vigorously sniffed the ground. The function of social sniffing appears to be similar to that of "group ceremonies" in wolves (Mech 1970) or "social rallies" in African hunting dogs (Estes and Goddard 1967; Creel and Creel 1995). That is, social sniffing among spotted hyenas appears to function as a mechanism to elevate and synchronize the arousal levels experienced by multiple individuals concurrently (Kruuk 1972; Holekamp et al. 2000). Thus, adult males often worked themselves into a highly excited state before they approached and threatened a female.

The finding that males bait females during the phases of her reproductive cycle when she is most likely to conceive suggests that males are able to detect when a female is in, or is approaching, estrus. Endocrine correlates of estrus have not been described in the spotted hyena, but plasma levels of estrogen, progesterone, and testosterone all appear to be much higher around the time of parturition than around the time of conception in spotted hyenas (Licht et al. 1992; Holekamp et al., unpublished data). Levels of excreted testosterone in feces also peak late in gestation in this species (Dloniak et al., in prep). Thus, absolute levels of these steroid hormones in females do not appear to play a significant role in stimulating baiting behavior by males. However, it remains

possible that the relative levels of some combination of steroid hormones, including androstenedione, might cue males that a female is approaching estrus.

Four hypotheses suggest possible adaptive functions of male attacks directed toward females in the spotted hyena. First, male hyenas might attack females to displace them from desired resources. This hypothesis predicts that baiting should occur in temporal association with feeding competition, and that males should at least occasionally be able to usurp food from females. Although 26% of observed baitings occurred at sites where ungulates were killed and devoured, no male attacks against females involved contests over food or any other resource. Furthermore, since immigrant male hyenas invariably defer to females during feeding competition (Kruuk 1972; Tilson and Hamilton 1984; Frank 1986b), we can promptly reject this hypothesis.

A second hypothesis suggests that aggressive behavior might allow males to test female receptivity (Beach 1976). If males are using aggressive acts to test female receptivity, then we would predict that responses by females to male attacks should vary with female reproductive condition, but we found no such variation, and baiting did not immediately precede the few successful copulations that we did observe. In many other mammalian species, males assess female reproductive state via investigation of chemical signals emitted by females (e.g. Johnston 1983), yet such assessment is generally not associated with attack behavior directed by the male at the female. Male *Crocuta* also frequently engage in a variety of non-aggressive behaviors that appear to give them information about a female's current reproductive state (see Chapter 3), such as

sniffing her body and recently excreted urine and feces which contain gonadal steroid hormones and their metabolites (Koretz 1992; S.M. Dloniak et al. & S.E. Glickman et al. unpublished data). Our observations demonstrate that male approaches are less likely to be met with aggression by the female if the male approaches non-aggressively. Therefore, risk of injury to both the male and the female is lower for these non-aggressive interactions. Because males should incur lower costs when they obtain information on female reproductive state via interactions with females that involve no aggression, we conclude that this functional explanation for baiting behavior seems unlikely.

Third, male attacks directed toward females might function in this species as a form of sexual coercion, or sexual harassment. That is, baiting behavior may function to increase the probability that a female will mate with a particular male at a time when she is likely to be fertile, and to decrease the probability that she will mate with other males at that time. As summarized by Smuts & Smuts (1993), this hypothesis predicts that male aggression toward females should occur frequently when females are most likely to conceive new litters, should occur infrequently when females are in other phases of their reproductive cycles, and should incur some cost to the female. These predictions are confirmed by our observations of male aggression toward females among *Crocuta*.

Clutton-Brock & Parker (1995) suggested three general forms of sexual coercion: (1) intimidation, where males punish females who refuse to mate and thus increase the probability of securing future matings, (2) forced copulation, where physical force is used to achieve intromission, and (3) harassment, where

male aggression imposes costs on females that increase the probability of immediate mating. Intimidation seems unlikely here considering that adult female *Crocuta* are approximately 10% larger than males (Matthews 1939; Wildon 1968; Kruuk 1972; Frank 1986b), and sport weapons that are effectively indistinguishable from those of males. Furthermore, we found that non-sires baited females at higher rates before than after conception, the opposite of the result predicted by the intimidation hypothesis. Forced copulation can most likely be ruled out altogether in this species in light of the monomorphic external genitalia of males and females. That is, the female's clitoris is elongated into a fully erectile pseudopenis, through which the urogenital canal extends, and through which the female urinates, copulates, and gives birth (Matthews 1939; Neaves et al. 1980; Frank et al. 1990). The bizarre genital morphology of the female makes rape impossible, and intromission by the male requires complete female cooperation.

Because intimidation and forced copulation seem unlikely in the spotted hyena, we suggest that male aggression toward females might function mainly to elevate stress levels in females or otherwise alter female reproductive endocrinology. Baiting of lactating females might induce stress that reduces milk production, and thus hasten the end of lactation. Stress is known to depress milk production in other mammals (e.g. dairy cows, *Bos taurus*: Ray et al. 1992; Tancin et al. 1995). Baiting of female hyenas during the early weeks of pregnancy might stress females when fetuses are highly vulnerable, and thus induce abortion (Wasser and Barash 1983). Although we currently have no

method for detecting abortions in free-living hyenas, we found that 13 of 20 observed copulations failed to result in the birth of a litter, and thus appeared to be infertile matings. Baiting during the period between weaning one litter and conceiving the next might either induce early abortions or alter the reproductive endocrinology of the non-pregnant female to bring her more rapidly into estrus. We can infer that baiting imposes costs on female *Crocuta* because females sometimes sustain wounds during baiting, females often fight back in ways that risk further injury, baiting appears stressful for female targets, and baiting occupies time and energy that females might otherwise spend resting, foraging, or caring for their cubs. However, the fact that sires were observed baiting females with whom they had recently mated provides evidence against this hypothesis, as does our observation that sires baited females at rates that did not differ before and after conception.

Finally, baiting might function to demonstrate some aspect of male fitness to prospective mates. This hypothesis predicts that aggressive behavior directed at females should vary among individual males, and that male reproductive success should vary with some parameter of male aggressive behavior. Although rates of baiting did not differ statistically significantly among individual males, there was a 15-fold difference between the minimum and maximum rates at which any individual male baited females. This, together with our results showing that individual males did vary significantly with respect to numbers of aggressive acts they directed toward females during each baiting, suggests that there are individual differences in baiting behavior among males, which might potentially

reflect male fitness. However, males did not appear to gain direct mating opportunities as a result of baiting. Males who sired the cubs of a target female did not bait her at greater rates or with greater intensity than did males who failed to sire cubs of that female. We might not expect to see as strong a relationship in this species between rates of baiting behavior and success at siring cubs as we do, for example, between association indices and success at siring cubs (Szykman et al. 2001). This is because most baitings involve a group of males, whereas only one or two males can potentially sire a female's litter.

The mechanism by which males are demonstrating their fitness to females might not be through repeated baitings, as measured by baiting rates, but through individual acts of aggression exhibited during baiting. That is, females might assess males based on some particular aspect of individual male behavior during baiting. Therefore, the hypothesis remains viable that males might demonstrate some aspect of their fitness to females during baiting. Here, sample sizes of cases in which we were able to reliably record all specific behavioral acts by all participating males were too small to yield much statistical power, so this matter remains unresolved.

In spotted hyenas, male aggression directed towards females remains a fascinating and mysterious phenomenon. The tight association between male attacks on females and a female's time to conception provides strong evidence for some role for male aggression in hyena sexual behavior. None of the hypotheses presented here satisfactorily explain all aspects of baiting behavior, although our data are most consistent with the hypothesis that male attacks on

females play a role in informing the female about some aspect of male fitness. Whatever the function of baiting, given the larger body size, peculiar genital morphology, and social dominance of female *Crocuta*, if male aggression towards females is related to sexual behavior, it probably functions in conjunction with female choice as a mode of sexual selection in this species.

Chapter 5

CONCLUSIONS

The inspiration for this dissertation was provided by the exciting findings of Holekamp and colleagues (1996), in which they determined that reproductive success varies significantly with social rank among free-living female spotted hyenas. High-ranking female hyenas begin breeding at younger ages, are more frequently able to support pregnancy and lactation concurrently, experience shorter intervals between litters, and their offspring are more likely to survive to adulthood than are those of lower-ranking females. The finding that social rank affects female reproductive success was not unique: in several gregarious mammalian species, high-ranking females enjoy greater reproductive success than do their lower-ranking counterparts (e.g. primates: Altmann et al. 1988; ungulates: Clutton-Brock et al. 1988; carnivores: Creel et al. 1992). But the rankrelated variation in reproductive output is extreme in *Crocuta*; the highest-ranking females in a single clan benefit from a six-fold greater lifetime reproductive success than do the lowest-ranking females (Holekamp et al. 1996; Holekamp and Smale 2000). These results motivated me to inquire what the males were doing with respect to mate choice and sexual behavior in this peculiar society.

When I first embarked on my study of mate choice and sexual behavior in spotted hyenas, I did not think that the males would be able to express their mate choices as straightforwardly as occurs in other polygynous animals in which

males are known to be selective (Verrell 1990; Berglund and Rosenqvist 1993). In general, male mammals in polygynous societies tend to be larger and more aggressive than females, and they usually compete intensely for access to breeding females (Darwin 1871; Clutton-Brock 1989). By contrast, adult breeding Crocuta males are smaller than females and socially subordinate to them. In addition, males generally exhibit only low levels of intrasexual aggression, and their courtship behavior is fairly subtle (Kruuk 1972; Frank 1986b). However, with such substantial variation among female hyenas in regard to their guality as mates, sexual selection theory predicts that male hyenas should be selective in their choice of mates (Gwynne 1981; Parker 1983; Petrie 1983; Owens and Thompson 1994). This study thus sought to examine these theoretical predictions regarding male mate choice in the spotted hyena. I used behavioral data collected in the field on a free-living population of spotted hyenas to address these predictions regarding male mate choice, and subsequently used information obtained from genetic paternity data to show a relationship between male mate choice behavior and male reproductive success. A second goal was to further elucidate the mating system of, and sexual behavior in, this species. Finally, I wanted to describe male-female interactions not previously reported in the literature, and to attempt to elucidate the functional significance of a subset of these.

We examined association patterns among male and female spotted hyenas in Chapter 2, male and female sexual behavior in Chapter 3, and male aggression towards females in Chapter 4. All of these behaviors were influenced

by female reproductive condition and, to varying extents, by male and female social rank. We found that male hyenas associate most closely, and in the greatest numbers, with females most likely to conceive (Chapter 2). Males also direct a greater number of affiliative behaviors toward females as females approach the time of conception (Chapter 3). Finally, males direct the greatest number of aggressive attacks toward females, and males attack females at the highest rates, during the periods when females are most likely, or are known, to conceive (Chapter 4).

Since morphological characteristics of female hyenas, and behavioral signals emitted by females, do not appear to vary with their reproductive condition, males presumably evaluate female reproductive state using olfactory cues. Such information obtained by males during investigatory behaviors directed toward females appears to influence male movement patterns. That is, males appear to use the female's reproductive condition, measured here as time to conception, in making decisions regarding whether or not to associate closely with a particular female at a given time. It is also possible that females exhibit other subtle signs reflecting their willingness to mate, including increased tolerance to the presence of males, which males may perceive and use in their decision-making processes.

Male hyenas were found to associate more closely with high-ranking than with low-ranking females (Chapter 2). However, effects of female social rank do not have as strong an influence on male affiliative behaviors as expected (Chapter 3). Instead, we found a significant interaction between a female's rank

and her age, whereby males appear to direct more affiliative behaviors towards older, low-ranking females than toward younger ones. Interestingly, female age did not influence male attention directed toward high-ranking females. Both of these results are consistent with known variation in life histories between highand low-ranking females. High-ranking females begin producing offspring soon after reaching reproductive maturity at 2 years of age (Holekamp et al. 1996; this study), whereas low-ranking females begin breeding at significantly older ages (Holekamp et al. 1996). Intersexual association patterns and data documenting affiliative behavior indicated that male hyenas can assess the relative reproductive value of individual females, and that males appear to allocate their own reproductive effort among clan females based on this assessment.

It is not known by what mechanism male hyenas are able to discriminate among females of various ranks. Males might be able to assess relative female ranks directly by observing dyadic agonistic interactions among females. It is reasonable to believe that males might be using the same mechanisms to understand rank relationships among females in their new clans that hyena cubs do when first learning their own social ranks. That is, in addition to participating in coalitions that reinforce aggression directed at lower-ranking conspecific targets, young spotted hyenas may learn their positions in the clan's social hierarchy by watching their mothers intervene on their behalf in fights with other clan members (Engh et al. 2000). It is less clear by what mechanism males are associating high rank with high reproductive value among females. Males might use female rank as an indirect measure of female reproductive success, much as male insects

and amphibians use female size as an indirect measure of female fecundity (Gwynne 1981; Verrell 1995). Alternatively, males might be able to recognize relatedness among natal hyenas. Males may discriminate those females who produce many offspring from those females who only produce a few by monitoring association patterns among natal animals (kin associate more closely than do non-kin: Holekamp et al. 1997), or via MHC odortype cues (Yamazaki et al. 1994; Todrank et al. 1998). Studies of kin recognition in *Crocuta* are currently in progress in the Holekamp lab (R.C. Van Horn and S.A. Wahaj, unpublished data).

Female social rank does not appear to influence male attacks on females (Chapter 4), suggesting that these attacks are sexually motivated behaviors directed at a female near her time of conception regardless of her long-term reproductive value. Most males present in the clan for several years bait females, and males of higher social rank bait females at higher rates than do lower-ranking males. It is possible that males of different ranks adopt different strategies to attract mates. This hypothesis is supported by the male-female association data. Both high- and middle-ranking males associate most closely with high-ranking females, but low-ranking males may either be less adept at assessing the reproductive value of clan females, or they may utilize different reproductive strategies than those used by high-ranking males, whose dominant status may often allow them to exclude lower-ranking males from access to high-value females.

The ability of male hyenas to access or monopolize receptive females should vary with the degree to which estrous cycles are synchronized among clan females. Data from Chapter 2 suggest that female reproductive synchrony is relatively low in our hyena study population, and that males should be able to monopolize females without incurring significant costs resulting from lost mating opportunities. However, two issues subsequently arise. First, under certain ecological conditions, up to nine females may be in estrus concurrently (Chapter 2), and thus males are forced to make decisions regarding in which female they should invest time and effort. Second, I suggest that it is precisely this time and effort necessary for males to persuade females to mate with them that requires males to make informed decisions about with whom to associate closely, even if females are never concurrently in estrus. At least for some males, this strategy of "consorting" with a particular female may be related to his chances of mating with her (see temporal patterns of intersexual association in Chapter 3).

The real challenge to the behavioral data presented in this dissertation is to unambiguously demonstrate that close intersexual associations and increased affiliative behavior by males directed at females represent mate choice by males, and that these measures truly reflect increased reproductive success for males. Evidence from other polygynous mammalian species indicates that consorting pairs are frequently observed to copulate, and that consorting males father most offspring (Packer and Pusey 1982; Bercovitch 1987; Small 1990). In this study, close and relatively exclusive intersexual associations are associated with paternity in spotted hyenas (Chapter 2), but the direction of the cause-and-effect

relationship involved remains unknown. That is, a male might make himself more attractive to a particular female by spending more time with her, and thus enhancing his own chances of siring her cubs. Alternatively, a male might spend more time with a female because she has already chosen him to sire her cubs. Clearly our data do not rule out the possibility that close intersexual associations in *Crocuta* reflect mate choice by females as well as by males.

I believe I have successfully shown that spotted hyenas are indeed fascinating subjects for the study of sexual behavior, particularly when their unique behaviors are considered in the context of sexual selection theory. On the one hand, hyenas represent a typical polygynous mammalian species in which males provide no parental care for their offspring, and females are expected to be selective in their choice of mates. However, whereas male-male competition is predicted in most polygynous species, male aggression toward other males is surprisingly low in *Crocuta* (Kruuk 1972, this study; Frank 1986b). Furthermore, the high variation in female mate quality observed in *Crocuta* provides male hyenas with a strong incentive for mate choice. In this study, I sought to elucidate sexual behavior in spotted hyenas and examine male behavior indicative of mate choice. Courtship and mating are unique in this species, and male hyena behavior appears to reflect some degree of choice for high-ranking, high-quality females.

There are several questions that emerge from this study and require additional investigation by future workers. Additional data need to be collected to determine more clearly the function of male baiting behavior. A new approach

might be to address the coordinated behavior among immigrant males when baiting. Determining whether individual males are cooperating with one another during baiting to increase their inclusive fitness, as might be reflected in levels of genetic relatedness among participating males, could shed further light on the adaptive function of this unusual behavior.

A next logical step would be to attempt to elucidate individual reproductive strategies and mate choice decisions among female spotted hyenas. Watching a single female allow two males to alternate mounting her, which we saw on two separate occasions (Chapter 3) gives observers the impression that the female is not doing much choosing. We even know from genetic paternity analyses that about 20% of twin litters are sired by multiple males (Engh et al. 2002). However, it is entirely possible that female hyenas have made their choices long before copulation occurs. In addition, it remains possible that female Crocuta can continue making mate-choice decisions after they have copulated with multiple males via cryptic choice (Eberhard 1996). Indeed, the bizarre and tortuous configuration of the female reproductive tract in this species (Frank et al. 1995b) suggests that female hyenas might be able to create competitive conditions for sperm between copulation and the point at which egg and sperm actually meet. Because females are socially dominant in *Crocuta*, and they possess masculinized genitalia that make copulation by force impossible, it is highly likely that females are running the mating game and making final choices regarding which males sire their offspring. Further study of female mate choice should nicely complement analyses of male mate choice in this species. That is,

understanding what a female is looking for in a mate, and determining the mechanisms by which she evaluates males and acts on her decisions, should yield further insight into male reproductive strategies.

LITERATURE CITED

- Altmann J (1974) Observational study of behavior: sampling methods. Behaviour 49:227-267
- Altmann J, Hausfater G, Altmann SA (1988) Determinants of reproductive success in savannah baboons, *Papio cynocephalus*. In: Clutton-Brock TH (ed) Reproductive Success. University of Chicago Press, Chicago, pp 403-418
- Andersson M (1982) Female choice selects for extreme tail length in a widowbird. Nature 299:818-820

Andersson M (1994) Sexual Selection. Princeton University Press, Princeton

Asa CS, Valdespino C (1998) Canid reproductive biology: an integration of proximate mechanisms and ultimate causes. American Zoologist 38:251-259

Bateman AJ (1948) Intrasexual selection in Drosophila. Heredity 2:349-368

- Beach FA (1976) Sexual attractivity, proceptivity, and receptivity in female mammals. Hormones and Behavior 7:105-138
- Beach FA, Dunbar IF, Buehler MG (1982) Sexual characteristics of female dogs during successive phases of the ovarian cycle. Hormones and Behavior 16:414-442
- Beach FA, LeBoeuf BJ (1967) Coital behavior in dogs. I. Preferential mating in the bitch. Animal behaviour 15:546-558

Beaver BV (1992) Feline behavior: A guide for veterinarians. WB Saunders Company, Philadelphia

Bercovitch FB (1987) Reproductive success in male savanna baboons.

Behavioral Ecology and Sociobiology 21:163-172

- Bercovitch FB (1991) Mate selection, consortship formation, and reproductive tactics in adult female savanna baboons. Primates 32:437-452
- Bercovitch FB (1995) Female cooperation, consortship maintenance, and male mating success in savanna baboons. Animal Behaviour 50:137-149
- Bercovitch FB, Sladky KK, Roy MM, Goy RW (1987) Intersexual aggression and male sexual activity in captive rhesus macaques. Aggressive Behavior 13:347-358
- Berger J (1983) Induced abortion and social factors in wild horses. Nature 303:59-61
- Berger J (1989) Female reproductive potential and its apparent evaluation by male mammals. Journal of Mammalogy 70:347-3358
- Berglund A, Rosenqvist G (1993) Selective males and ardent females in pipefishes. Behavioral Ecology and Sociobiology 32:331-336
- Bertram BCR (1975) Social factors influencing reproduction in lions. Journal of Zoology 177:463-482
- Borgia G (1985) Bower quality, number of decorations and mating success of male satin bowerbirds (*Ptilonorhynchus violaceus*): An experimental analysis. Animal Behaviour 32:266-271

- Boydston EE, Morelli TL, Holekamp KE (2001) Sex differences in territorial behavior exhibited by the spotted hyena (Hyaenidae, *Crocuta crocuta*). Ethology 107:369-385
- Bradbury JW, Gibson RM (1983) Leks and mate choice. In: Bateson P (ed) Mate Choice. Cambridge University Press, Cambridge, pp 109-138
- Bronson FH (1989) Mammalian reproductive biology. University of Chicago Press, Chicago
- Burley N, Moran N (1979) The significance of age and reproductive experience in the mate preferences of feral pigeons, *Columbia livia*. Animal Behaviour 27:686-698
- Bygott JD, Bertram BC, Hanby JP (1979) Male lions in coalitions gain reproductive advantages. Nature 282:839-41
- Cairns SJ, Schwager SJ (1987) A comparison of association indices. Animal Behaviour 35:1454-1469
- Campagna C, LeBoeuf BJ (1988) Reproductive behaviour of southern sea lions. Behaviour 105:233-261
- Caro TM (1994) Cheetahs of the Serengeti Plains. University of Chicago Press, Chicago
- Chakraborty R, Meaher T, Smouse P (1988) Parentage analysis with genetic markers in natural populations. I. The expected proportion of offspring with unambiguous paternity. Genetics 118:527-536
- Clutton-Brock TH (1989) Mammalian mating systems. Proceedings of the Royal Society of London, Series B 236:339-372

Clutton-Brock TH, Albon SD, Guinness FE (1982) Red Deer: Behavior and Ecology of Two Sexes. University of Chicago Press, Chicago

- Clutton-Brock TH, Albon SD, Guinness FE (1988) Reproductive success in male and female red deer. In: Clutton-Brock TH (ed) Reproductive Success. University of Chicago Press, Chicago, pp 325-343
- Clutton-Brock TH, Parker GA (1995) Sexual coercion in animal societies. Animal Behaviour 49:1345-1365
- Clutton-Brock TH, Vincent ACJ (1991) Sexual selection and the potential reproductive rates of males and females. Nature 351:58-60
- Connor RC, Smolker RA, Richards AF (1992a) Dolphin coalitions and alliances. In: Harcourt AH, de Waal FBM (eds) Coalitions and Alliances in Humans and Other Animals. Oxford University Press, Oxford, pp 415-443
- Connor RC, Smolker RA, Richards AF (1992b) Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). Proceedings of the National Academy of Science 89:987-990
- Côte IM, Hunte W (1989) Male and female mate choice in the redlip blenny: Why bigger is better. Animal Behaviour 38:78-88
- Cox CR, LeBoeuf BJ (1977) Female incitation of male competition: Mechanism in sexual selection. American Naturalist 111:317-335
- Creel S, Creel N, Wildt DE, Monfort SL (1992) Behavioral and endocrine mechanisms of reproductive suppression in Serengeti dwarf mongooses. Animal Behaviour 43:231-245

- Creel S, Macdonald D (1995) Sociality, group-size, and reproductive suppression among carnivores. Advances in the Study of Behavior 24:203-257
- Creel SR, Creel NM (1995) Communal hunting and pack size in African wild dogs Lycaon pictus. Animal Behaviour 50:1325-1339

Crews D (1975) Psychobiology of reptilian reproduction. Science 189:1059-1065

Dagg AI, Taub A (1970) Flehmen. Mammalia 34:686-695

- Darwin C (1871) The Descent of Man and Selection in Relation to Sex. John Murray, London
- Davies NB (1991) Mating systems. In: Krebs JR, Davies NB (eds) Behavioural Ecology, an Evolutionary Approach, 3rd edn. Blackwell Scientific Publications, Oxford, pp 263-294
- Derix RRWM, van Hooff JARAM (1995) Male and female partner preferences in a captive wolf pack (*Canis lupus*): Specificity versus spread of sexual attention. Behaviour 132:128-148
- Dewsbury DA (1972) Patterns of copulatory behavior in male mammals. The Quarterly Review of Biology 47:1-33
- Doving KB, Trotier D (1998) Structure and function of the vomeronasal organ. Journal of Experimental Biology 201:2913-2925
- East ML, Hofer H, Wickler W (1993) The erect penisis a flag of submission in a female-dominated society: Greetings in Serengeti spotted hyenas. Behavioral Ecology and Sociobiology 33:355-370
- Eberhard WG (1996) Female Control: Sexual Selection by Cryptic Female Choice. Princeton University Press, Princeton

- Emlen ST, Oring LW (1977) Ecology, sexual selection and the evolution of mating systems. Science 197:215-223
- Engh AL, Esch K, Smale L, Holekamp KE (2000) Mechanisms of matenal rank 'inheritance' in the spotted hyaena, *Crocuta crocuta*. Animal Behaviour 60:323-332
- Engh AL, Van Horn RC, Funk SM, Scribner KT, Bruford MW, Szykman M, Smale L, Holekamp KE (2002) Reproductive skew among males in a female dominated mammalian society. Behavioral Ecology In press
- Estes RD (1972) The role of the vomeronasal organ in mammalian reproduction. Mammalia 36:315-341
- Estes RD, Goddard J (1967) Prey selection and hunting behavior of the African wild dog. Journal of Wildlife Management 32:52-76

Ewer RF (1973) The Carnivores. Cornell University Press, Ithaca

- Forsberg J (1987) A model for male mate discrimination in butterflies. Oikos 49:46-54
- Frank LG (1986a) Social organization of the spotted hyaena (*Crocuta crocuta*). 1. Demography. Animal Behaviour 34:1500-1509

Frank LG (1986b) Social organization of the spotted hyaena *Crocuta crocuta*. 2. Dominance and reproduction. Animal Behaviour 34:1510-1527

Frank LG (1997) Evolution of genital masculinization: Why do female hyaenas have such a large 'penis'? Trends in Ecology and Evolution 12:58-62

- Frank LG, Glickman SE (1994) Giving birth through a penile clitoris: Parturition and dystocia in the spotted hyena (*Crocuta crocuta*). Journal of Zoology 234:659-665
- Frank LG, Glickman SE, Powch I (1990) Sexual dimorphism in the spotted hyena (*Crocuta crocuta*). Journal of Zoology 221:308-313
- Frank LG, Holekamp KE, Smale L (1995a) Dominance, demography, and
 reproductive success of female spotted hyenas. In: Sinclair ARE, Arcese
 P (eds) Serengeti II: Dynamics, management, and conservation of an
 ecosystem. University of Chicago Press, Chicago, pp 364-384
- Frank LG, Weldele ML, Glickman SE (1995b) Masculinization costs in hyaenas. Nature 377:584-585
- Gibson RM, Langen TA (1996) How do animals choose their mates? Trends in Ecology and Evolution 11:468-470
- Golding RR (1969) Birth and development of spotted hyaenas *Crocuta crocuta* at the University of Ibadan Nigeria. International Zoo Yearbook 9:93-95
- Goodall J (1986) The chimpanzees of Gombe: Patterns and Behavior. Harvard University Press, Cambridge

Grimpe G (1916) Hyänologische Studien. Zool Anz 48:49-61

- Gross MR, Shine R (1981) Parental care and mode of fertilization in ectothermic vertebrates. Evolution 35:775-793
- Gwynne DT (1981) Sexual difference theory: Mormon crickets show role reversal in mate choice. Science 213:779-780

Halliday TR (1983) The study of mate choice. In: Bateson PPG (ed) Mate Choice. Cambridge University Press, Cambridge, pp 3-32

- Hamilton WJ, Tilson RL, Frank LG (1986) Sexual monomorphism in spotted hyenas, *Crocuta crocuta*. Ethology 71:63-73
- Hausfater G (1975) Dominance and reproduction in baboons. Contributions in Primatology 7:1-150
- Hayssen V, van Tienhoven A, van Tienhoven A (1993) Adell's Patterns of Mammalian Reproduction. Cornell University Press, Ithaca
- Henschel JR, Skinner JD (1987) Social relationships and dispersal patterns in a clan of spotted hyaenas *Crocuta crocuta* in the Kruger National Park. South African Journal of Zoology 22:18-24
- Hill DA (1987) Social relationships between adult male and female rhesus macaques. 1. Sexual consortships. Primates 28:439-456
- Hill GE (1990) Female house finches prefer colourful males: Sexual selection for a condition-dependent trait. Animal Behaviour 40:563-572
- Hofer H, East ML (1993) The commuting system of Serengeti spotted hyaenas: How a predator copes with migratory prey. 3. Attendance and maternal care. Animal Behaviour 46:575-589
- Hofer H, East ML (1995) Virilized sexual genitalia as adaptations of female spotted hyaenas. Revue Suisse De Zoologie 102:895-906
- Hoikkala A, Aspi J, Suvanto L (1998) Male courtship song frequency as an indicator of male genetic quality in an insect species, *Drosophila montana*.
 Proceedings of the Royal Society London, Series B 265:503-508

Holekamp KE, Boydston EE, Smale L (2000) Group travel in social carnivores. In: Boinski S, Garber PA (eds) On the move: How and why animals travel in groups. University of Chicago Press, Chicago, pp 587-627

- Holekamp KE, Cooper SM, Katona CI, Berry NA, Frank LG, Smale L (1997) Patterns of association among female spotted hyenas (*Crocuta crocuta*). Journal of Mammalogy 78:55-64
- Holekamp KE, Smale L (1990) Provisioning and food sharing by lactating spotted hyenas, *Crocuta crocuta* (Mammalia, Hyaenidae). Ethology 86:191-202
- Holekamp KE, Smale L (1993) Ontogeny of dominance in free-living spotted hyaenas: Juvenile rank relations with other immature individuals. Animal Behaviour 46:451-466

Holekamp KE, Smale L (1998) Dispersal status influences hormones and behavior in the male spotted hyena. Hormones and Behavior 33:205-216

Holekamp KE, Smale L (2000) Feisty females and meek males. In: Wallen K, Schneider JE (eds) Reproduction in Context: Social and Environmental Influences on Reproduction. MIT Press, Cambridge, pp 257-285

- Holekamp KE, Smale L, Szykman M (1996) Rank and reproduction in the female spotted hyaena. Journal of Reproduction and Fertility 108:229-237
- Hosmer DW, Lemeshow S (2000) Applied Logistic Regression, Second edn. John Wiley & Sons, Inc., New York

Johnston RE (1983) Chemical signals and reproductive behavior. In: Vandenbergh JG (ed) Pheromones and Reproduction in Mammals. Academic Press, New York, pp 3-37

- Karino K (1995) Male-male competition and female mate choice through courtship display in the territorial damselfish *Stegastes nigricans*. Ethology 100:126-138
- King C (1989) The Natural History of Weasels and Stoats. Comstock Publishing Associates, Ithaca
- Komers PE, Messier F, Flood PF, Gates CG (1994) Reproductive behavior of male wood bison in relation to progesterone level in females. Journal of Mammalogy 75:757-765
- Koretz BK (1992) Excretion of testosterone, estradiol, and progesterone in the feces of spotted hyena (*Crocuta crocuta*). Undergraduate honors thesis, University of California, Berkeley
- Kruuk H (1972) The Spotted Hyena: A Study of Predation and Social Behavior. University of Chicago Press, Chicago
- Kvarnemo C, Ahnesjo I (1996) The dynamics of operational sex ratios and competition for mates. Trends in Ecology and Evolution 11:404-408
- Kvarnemo C, Forsgren E, Magnhagen C (1995) Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. Animal Behaviour 50:1455-1461
- LeBoeuf BJ, Mesnick S (1990) Sexual behavior of male northern elephant seals: I. Lethal injuries to adult females. Behaviour 116:143-162

Legendre P, Legendre L (1998) Numerical Ecology, Second edn. Elsevier, Amsterdam

Libants S, Olle E, Oswald K, Scribner KT (2000) Microsatellite loci in the spotted hyena *Crocuta crocuta*. Molecular Ecology 9:1443-1445 Licht P, Frank LG, Pavgi S, Yalcinkaya TM, Siiteri PK, Glickman SE (1992) Hormonal correlates of masculinization in female spotted hyaenas (*Crocuta crocuta*). 2. Maternal and fetal steroids. Journal of Reproduction and Fertility 95:463-474

- Lindburg DG (1983) Mating behavior and estrus in the Indian rhesus monkey. In: Seth PK (ed) Perspectives in Primate Biology. Today and Tomorrow, New Delhi, pp 45-61
- Lindeque M (1981) Reproduction in the spotted hyena *Crocuta crocuta* (Erxleben). MSc thesis, University of Pretoria, Pretoria
- Linklater WL, Cameron EZ, Minot EO, Stafford KJ (1999) Stallion harassment and the mating system of horses. Animal Behaviour 58:295-306
- Littlejohn MJ, Loftus-Hills JJ (1968) An experimental evaluation of premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). Evolution 22:659-663
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655

Matthews LH (1939) Reproduction in the spotted hyena *Crocuta crocuta* (Erxleben). Philosophical Transactions Royal Society, Series B 230:1-78

McKinney F, Derickson SR, Mineau P (1983) Forced copulation in waterfowl. Behaviour 86:250-294
Meagher TR (1986) Analysis of paternity within a natural population of
Chemaelirium luteum. I. Identification of most-likely male parents.
American Naturalist 128:199-215

Mech LD (1970) The Wolf: The Ecology and Behavior of an Endangered Species. Natural History Press, Garden City

Melnick DJ, Pearl MC (1987) Cercopithecines in multimale groups: Genetic diversity and population structure. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) Primate Societies. University of Chicago Press, Chicago, pp 318-329

- Mesnick SL, Le Boeuf BJ (1991) Sexual behavior of male northern elephant seals: II. Female response to potentially injurious encounters. Behaviour 117:262-280
- Mills MGL (1990) Kalahari Hyenas: The comparative behavioral ecology of two species. Chapman and Hall, London
- Mitani JC (1985) Mating behaviour of male orangutans in the Kutai Game Reserve, Indonesia. Animal Behaviour 33:392-402
- Moehlman PD (1989) Intraspecific variation in canid social systems. In: Gittleman JL (ed) Carnivore Behavior, Ecology, and Evolution, vol 1. Cornell University Press, Ithaca, pp 143-163
- Neaves WB, Griffin JE, Wilson JD (1980) Sexual dimorphism of the phallus in spotted hyena (*Crocuta crocuta*). Journal of Reproduction and Fertility 59:506-512

- Oring LW, Reed JM, Colwell MA, Lank DB, Maxson SJ (1991) Factors regulating annual mating success and reproductive success in spotted sandpipers (*Actitis macularia*). Behavioral Ecology and Sociobiology 28:433-442
- Owens IPF, Thompson DBA (1994) Sex differences, sex ratios and sex roles. Proceedings of the Royal Society of London, Series B 258:93-99
- Packer C, Pusey AE (1982) Cooperation and competition within coalitions of male lions: kin selection or game theory. Nature 296:740-742
- Packer C, Pusey AE (1983) Male takeovers and female reproductive parameters: a simulation of oestrus synchrony in lions (*Panthera leo*). Animal behaviour 31:334-40
- Parker GA (1983) Mate quality and mating decisions. In: Bateson P (ed) Mate Choice. Cambridge University Press, Cambridge, pp 141-166
- Parker GA, Baker RR, Smith VGF (1972) The origin and evolution of gamete dimorphism and the male-female phenomenon. Journal of Theoretical Biology 36:529-553
- Petrie M (1983) Mate choice in role-reversed species. In: Bateson P (ed) Mate Choice. Cambridge University Press, Cambridge, pp 167-179
- Ptacek MB (2000) The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. Behavioural Processes 51:111-134
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258-275

Rasa OAE (1977) The ethology and sociology of the dwarf mongoose (*Helogale undulata rufula*). Z Tierpsychol 43:337-406

- Ray DE, Halbach TJ, Armstrong DV (1992) Season and lactation number effects on mild production of dairy cattle in Arizona. Journal of Dairy Science 75:2976-2983
- Real L (1990) Search theory and mate choice: I. Models of single-sex discrimination. American Naturalist 136:376-404
- Robinson JG (1982) Intrasexual competition and mate choice in primates.

American Journal of Primatology Supplement 1:131-144

- Sargent RC, Gross MR, den Berghe EP (1986) Male mate choice in fishes. Animal Behaviour 34:545-550
- Schaller GB (1972) The Serengeti Lion: A Study in Predator-Prey Relations. University of Chicago Press, Chicago

Schneider KM (1926) Über Hyänenzucht. Die Pelztierzucht 2:1-4, 9-11, 12-14

Schwagmeyer PL (1995) Searching today for tomorrow's mates. Animal Behaviour 50:759-767

Schwagmeyer PL, Parker GA (1990) Male mate choice as predicted by sperm competition in thirteen-lined ground squirrels. Nature 348:62-64

Seyfarth RM (1978a) Social relationships among adult male and female baboons. I. Behavior during sexual consortships. Behaviour 64

Seyfarth RM (1978b) Social relationships among adult male and female baboons. II. Behavior throughout the female reproductive cycle. Behaviour 64

- Silk JB, Boyd R (1983) Cooperation, competition, and mate choice in matrilineal macaque groups. In: Wasser SK (ed) Social Behavior of Female Vertebrates. Academic Press, New York, pp 315-347
- Smale L, Frank LG, Holekamp KE (1993) Ontogeny of dominance in free-living spotted hyaenas: Juvenile rank relations with adult females and immigrant males. Animal Behaviour 46:467-477
- Smale L, Nunes S, Holekamp KE (1997) Sexually dimorphic dispersal in mammals: patterns, proximal causes, and consequences. Advances in the Study of Behavior 26:181-250
- Small MF (1990) Consortships and conceptions in captive rhesus macaques (*Macaca mulatta*). Primates 31:339-350
- Smuts BB (1983) Spatial relationships between adult male and female olive baboons. In: Hinde RA (ed) Primate Social Relationships. Sinauer Associate, Inc., Sunderland, pp 262-266
- Smuts BB (1985) Sex and Friendship in Baboons. Harvard University Press, Cambridge
- Smuts BB (1987a) Gender, aggression, and influence. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) Primate Societies. University of Chicago Press, Chicago, pp 400-412
- Smuts BB (1987b) Sexual competition and mate choice. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) Primate Societies. University of Chicago Press, Chicago, pp 385-399

Smuts BB, Smuts RW (1993) Male aggression and sexual coercion of females in nonhuman primates and other mammals: Evidence and theoretical implications. Advances in the Study of Behavior 22:1-63

Solomon NG (1993) Body size and social preferences of male and female prairie voles, *Microtus ochrogaster*. Animal Behaviour 45:1031-1033

Soltis J, Mitsunaga F, Shimizu K, Yanagihara Y, Nozaki M (1997) Sexual selection in Japanese macaques: I. Female mate choice or male sexual coercion? Animal Behaviour 54:725-736

- Stammback E (1987) Desert, forest, and montane baboons: Multilevel societies. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT (eds) Primate Societies. University of Chicago Press, Chicago, pp 112-120
- Sterck EHM, Watts DP, van Schaik CP (1997) The evolution of female social relationships in nonhuman primates. Behavioral Ecology and Sociobiology 41:291-309
- Stern BR, Smith DG (1984) Sexual behaviour and paternity in three captive groups of rhesus monkeys (*Macaca mulatta*). Animal Behaviour 32:23-32
- Stone GN (1995) Female foraging responses to sxual harassment in the solitary bee *Anthophora plumipes*. Animal Behaviour 50:405-412

Systat (1998) Systat 8.0: Statistics. SPSS, Inc., Chicago

Szykman M, Engh AL, Van Horn RC, Funk SM, Scribner KT, Holekamp KE (2001) Association patterns among male and female spotted hyenas (*Crocuta crocuta*) reflect male mate choice. Behavioral Ecology and Sociobiology 50:231-238

- Tancin V, Harcek L, Broucek J, Uhrincat M, Mihina S (1995) Effect of suckling during early lactation and changeover to machine milking on plasma oxytoci and cortisol levels and milking characteristics in Holstein cows.
 Journal of Dairy Research 62:249-256
- Tardiff SD (1997) The bioenergetics of prental behavior and the evolution of alloparental care in marmosets and tamarins. In: Solomon NG, French JA (eds) Cooperative Breeding in Mammals. Cambridge University Press, Cambridge, pp 11-33
- Thompson EA (1975) The estimation of pairwise relationships. Annals of HumanGenetics 39:173-188
- Thornhill R, Gwynne DT (1986) The evolution of sexual differences in insects. American Scientist 74:382-389
- Tilson RL, Hamilton WE (1984) Social dominance and feeding patterns of spotted hyaenas. Animal Behaviour 28:52-59
- Todrank J, Heth G, Johnston RE (1998) Kin recognition in golden hamsters: evidence for kinship odours. Animal Behaviour 55:377-386
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual Selection and the Descent of Man. Aldine, Chicago, pp 136-179
- Vargas A, Anderson SH (1998) Black-footed ferret (*Mustela nigripes*) behavioral development: Aboveground activity and juvenile play. Journal of Ethology 16:29-41