BEHAVIORAL AND GENETIC CONSEQUENCES OF DISPERSAL IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

By

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ABSTRACT

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Female spotted hyenas (*Crocuta crocuta*) remain in their natal group, or clan, but males disperse after puberty. Immigrants sire virtually all cubs so dispersal confers reproductive success, but dispersers pay potentially high costs; they risk severe aggression and lose all social rank. Immigrants become subordinate to all members of their new clan and remain subordinate to all natal clan-mates, even their own cubs. Given the costs of dispersal, it seems unlikely that males would disperse more than once (i.e., engage in secondary dispersal). I developed methods to estimate the ages of spotted hyenas and demonstrated that 7 of 41 immigrants were secondary dispersers.

Previous authors demonstrated that some males sired cubs before secondarily dispersing; others had not. There are two explanations for why secondary dispersal by male spotted hyenas is not contingent on reproductive success or failure; both explanations rely on errors in kin recognition. If sires failed to recognize their offspring,

sires might underestimate their reproductive success and disperse in an attempt to reproduce. Alternatively, if immigrants overestimated their reproductive success by misclassifying unrelated cubs as their own, males might secondarily disperse to avoid inbreeding when there was no risk of inbreeding. To assess errors in kin recognition I compared interactions between cubs and sires to their interactions with unrelated animals. Cubs directed lower rates of high intensity aggression towards their sires than towards control males and cubs associated more closely with their sires than with control males. Sires associated more closely with their own offspring than with others. Biases in behavior of sires and cubs suggest that they recognized one another. Secondary dispersal by male spotted hyenas was therefore not driven by errors in kin recognition.

Kin selection has been invoked to explain cooperation and group living among spotted hyenas. Kin must be present to provide opportunities for kin selection, but I used genetic estimates of relatedness among spotted hyenas to show that, on average, there are low levels of relatedness among clan-mates. Therefore it seems unlikely that clan-level cooperation is maintained by kin selection.

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My family may not understand why I do what I do, but they support me anyway. I owe a debt to Louie, although I've forgotten much that I learned from the big yellow dog. My life has been enormously enriched by the fantastic friendship, strong support, and everlasting encouragement of Sofia Wahaj, Cindy Wei, and Kathleen Kay. I resisted their efforts, but I thank these individuals for saving me from myself. I also thank Eva-Maria Muecke and Mary Martin, Megan Mahoney for chickens, Steph Dloniak for quick

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

Dispersal is the permanent relocation of an animal from one area or social unit to another, with natal (primary) dispersal involving departure from the animal's birthplace (Greenwood 1980). Successful dispersal in gregarious mammals entails not only emigration from one social unit, but also integration into another social unit. My dissertation evaluates the consequences of the dispersal process, and subsequent reproduction, in the spotted hyena (*Crocuta crocuta*) by integrating behavioral and molecular genetic data.

Spotted hyenas are large carnivores that live in stable social groups, or clans (Kruuk 1972). Each clan contains multiple adult females and their young, and at least one adult immigrant male. Hyena society is rigidly organized: each animal has a position in the clan's linear dominance hierarchy (Kruuk 1972; Tilson & Hamilton 1984; Frank 1986b; Smale et al. 1993). This position is not earned via aggressive competition, but is acquired through maternal rank inheritance whereby an individual achieves rank just below that of its mother (Holekamp & Smale 1991; Holekamp & Smale 1993; Engh et al. 2000). Social rank is important in this species because it determines an individual's priority

of access to food (Henschel & Skinner 1987; Mills 1990; Frank et al. 1995).

Dispersal in spotted hyenas is strongly male-biased, so that males leave their place of origin while females do not (Frank 1986b; Hofer & East 1993b; Smale et al. 1997; East & Hofer 2001). This pattern occurs in most mammals, but the social environment of spotted hyenas intensifies the pressures of natural selection normally acting on mammalian dispersers. The priority of access to food assured a male by his rank in his natal clan is lost when he disperses, because he becomes subordinate to every animal born in his new clan (Henschel & Skinner 1987; Smale et al. 1993; Smale et al. 1997; Holekamp & Smale 1998). Thus, a successful immigrant has the worst access to food of any animal in his new clan, until another immigrant joins the clan and becomes the lowest-ranking clan member; the only means by which an immigrant male can move up in rank is through attrition of the males who successfully immigrated into that group before him (Smale et al. 1997). Those prior immigrants sometimes direct intense aggression against the potential immigrant, while resident females often treat him with relative indifference (Boydston et al. 2001). Virtually all males emigrate, but most immigration attempts fail (Smale et al. 1997). Even if a male succeeds in

joining a clan, he is not assured of a reproductive payoff. On average an immigrant must remain in a clan for approximately two years before he sires his first offspring (Engh et al. 2002; East et al. 2003), and many males spend years of their lives in a clan without ever achieving measurable reproductive success (Engh et al. 2002). Dispersal among spotted hyenas thus exacts both a heavy social toll and high energetic cost, but does not guarantee a fitness benefit for dispersers. In spite of the apparent high costs of dispersal among spotted hyenas, it is ubiquitous and has several far-reaching implications for the behavioral ecology of spotted hyenas. Each chapter of this dissertation examines one such implication.

OVERVIEW OF CHAPTERS

Secondary dispersal is dispersal that occurs after the completion of natal dispersal (Pusey & Packer 1987). If a male spotted hyena were to undertake secondary dispersal, he would run a gauntlet of aggressive encounters with resident immigrant males (Smale et al. 1997) and survive a lengthy period of low social status, only to subsequently disperse again and begin the process anew: it would seem unlikely that males would undertake secondary dispersal. However, prior telemetry data from radio-collared animals suggested that some male spotted hyenas do secondarily disperse (Smale et al. 1997). In Chapter Two I develop a technique to estimate the ages of successful immigrant males and assess the relative frequency of secondary dispersal by comparing the estimated ages of immigrants into one clan of spotted hyenas to the known ages of emigrants from that clan. I demonstrate that a sizeable minority of immigrants undertook secondary dispersal. In Chapter Two I also illustrate that the age at which primary dispersal occurs varies widely. This variation, and the occurrence of secondary dispersal, means that potential immigrants into a clan are not a homogenous group of animals; rather they are a mixture of young, naive individuals and mature, experienced individuals. The work presented in Chapter Two has recently appeared in the *Journal of Mammalogy* (Van Horn et al. 2003b).

Many male spotted hyenas successfully transfer from one clan to another but subsequently fail to successfully reproduce (Engh et al. 2002). Dispersal does, however, greatly increase the likelihood that a male spotted hyena will reproduce. If he sires progeny, an immigrant male spotted hyena finds himself in the circumstance, unusual among vertebrates, of being socially subordinate to his own offspring (Smale et al. 1993; Smale et al. 1997; Holekamp & Smale 1998; East & Hofer 2001). Paternal nepotism, the

preferential treatment of offspring by a male, has been investigated in several species of mammals, but filial nepotism, the preferential treatment of a parent by offspring, has not been well studied other than in the context of cooperative breeding. In Chapter Three I illustrate that filial nepotism among spotted hyenas is more apparent during interactions between sires and cubs than is paternal nepotism. Chapter Three has been accepted pending minor revision at *Ethology* (Van Horn et al. 2003c).

Because nearly all spotted hyenas are the offspring of males born elsewhere (Engh et al. 2002), and because most males appear to disperse across few clan borders prior to reproducing (Smale et al. 1997; Boydston 2001), most gene flow occurs across few territorial boundaries (i.e., clan borders) and many spotted hyenas probably have close paternal kin in adjacent or nearby clans. Paternal gene flow within a clan is restricted by several aspects of the mating system of spotted hyenas. First, mate fidelity does not occur among either males or females in this species and second, there is low reproductive skew among both males and females relative to that observed in other social mammals (Engh et al. 2002; East et al. 2003). Third, females strongly prefer adult immigrant males as mates rather than adult natal males (Engh et al. 2002). In Chapter Four I

describe how low paternal gene flow within a clan, but high paternal gene flow among clans, results in low genetic relatedness within clans and little reduction in relatedness among clans. I highlight the implication of these patterns of relatedness with respect to cooperation among spotted hyenas: kin selection cannot explain clan-level cooperation in risky defense of resources against intra- and inter-specific competitors. Spotted hyenas exhibit nepotism in many contexts (e.g., Mills 1985), even towards their low-ranking sires, but kinship is not always enough to ensure cooperation. Chapter Four is in press at *Molecular Ecology* (Van Horn et al. 2003a).

Finally, three functional hypotheses have been proposed to explain sex-biased dispersal behavior in vertebrates (e.g., Dobson & Jones 1985). These hypotheses focus on competition for mates, competition for resources, and inbreeding avoidance. Neither mate competition nor resource competition appear to drive dispersal by male hyenas (Kruuk 1972; Mills 1990; Henschel & Skinner 1991; Hofer & East 1993a; Smale et al. 1997; Boydston et al. 2001) because males do not move to areas with fewer competitors, and dispersal decreases their access to food. Dispersal among mammals does not necessarily reduce opportunities for inbreeding (e.g., Gompper et al. 1998; Winters & Waser

2003) and I show in Chapters Three and Four that dispersal is not always necessary to avoid inbreeding. In Chapter Three I demonstrate that sires and their offspring discriminate each other from unrelated animals. This ability should also allow them to avoid incest. In Chapter Four I find that many pairs of unrelated hyenas are born within a clan, and the results presented in Chapter Three and by Wahaj et al. (2003) suggest that these unrelated animals can categorize each other as unrelated and therefore as potential mates. Mating within these unrelated pairs would permit inbreeding avoidance without dispersal. Nevertheless, the reproductive success of male spotted hyenas remains extremely low until after they disperse (Engh et al. 2002). During mate choice, female spotted hyenas ignore unrelated, reproductively mature, natal males and prefer to mate with immigrant males. The function of this preference remains unknown.

CHAPTER 2

AGE ESTIMATION AND DISPERSAL IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

INTRODUCTION

Age influences many aspects of mammalian biology, including physiology (Blom et al. 1994), epidemiology (Mills et al. 1999), and behavior (Bernstein & Ehardt 1985). Because anthropogenic disturbances may alter age-specific mortality and population structure (e.g., Hofer et al. 1993), the ability to estimate the ages of animals can facilitate their conservation. In addition, age data can enhance understanding of mammalian evolution, because age itself may be an important parameter in evolutionary processes such as sexual selection (Brooks & Kemp 2001).

Counts of dental annuli frequently are used to estimate the ages of mammals, including carnivores (Spinage 1973; Driscoll et al. 1985; Fandos et al. 1993; Landon et al. 1998). Annuli were used by van Jaarsveld et al. (1987) to estimate the ages of spotted hyenas (*Crocuta crocuta*) living at relatively high latitudes in southern Africa. These age estimates have been used in analyses of growth and development (van Jaarsveld et al. 1987; van Jaarsveld et al. 1988). However, formation of annuli may be irregular in *C. crocuta* (Lindeque & Skinner 1984) and other carnivores inhabiting constant environments, as is often

the case in equatorial regions. Also, this method requires tooth extraction, which may reduce future feeding efficiency of the subject (Van Valkenburgh 1988; Van Valkenburgh 1999).

Tooth-wear data represent another potential basis for age estimation in live African carnivores (Smuts et al. 1978; Stander 1997). Such data can be collected less intrusively than data on annuli, although both require immobilization of subjects. Kruuk (1972), in his seminal work on spotted hyena behavior and ecology, had no data from known-age animals but assigned individuals to 1 of 5 relative age classes based on wear of the third lower premolar (p3). Lindeque and Skinner (1984) later used the surface area of p3 to distinguish 7 relative age classes among South African *C. crocuta*. Relationships between dental morphology and age in captive spotted hyenas were described by Binder and Van Valkenburgh (2000), but the diet of captive animals is softer and probably more nutritious than that of free-living *C. crocuta* (Berger et al. 1992), so these relationships may differ among captive and wild populations as well as among wild populations with different diets. The teeth of young animals are more pointed and therefore wear more rapidly than the teeth of older animals: tooth wear tends to follow a negative exponential curve (Spinage 1973). Thus tooth wear criteria may overestimate the ages of young animals and underestimate the ages of older animals Spinage (1973) and model validation is particularly important. Known-age data from free-ranging *C. crocuta* have not been used to build or validate any age-estimation models, although such validation is desirable (e.g., Harris et al. 1992; Oosthuizen & Bester 1997; Gipson et al. 2000). The ability to estimate absolute age of spotted hyenas would facilitate the investigation of biological phenomena dependent on maturation, such as dispersal.

Natal, or primary, dispersal is the complete and permanent departure of an individual from its birthplace (Greenwood 1980). Secondary dispersal is any dispersal movement occurring after natal dispersal (Pusey & Packer 1987) and is relatively common among small mammals (reviewed as 'transfer' dispersal in Cockburn 1992) and some primates (reviewed in van Noordwijk & van Schaik 2001). With the exception of primates, it is considered uncommon among large mammals (Sinclair 1992). However, the extent to which it occurs among carnivores is currently unknown.

Female spotted hyenas rarely disperse from their social group, or clan, which includes adult natal females and

their offspring, and 1 to several adult immigrant males (Holekamp et al. 1993). However, nearly every male eventually emigrates and assumes low rank in another clan (Frank 1986b; Henschel & Skinner 1987; Hofer & East 1993b; Smale et al. 1997; Holekamp & Smale 1998; East & Hofer 2001) following puberty at about 24 months of age (Matthews 1939). Natal dispersal appears necessary for males to achieve reproductive success (Engh et al. 2002). Before both primary and secondary dispersal, males use the range of their current clan as a secure base from which to explore (Smale et al. 1997; Boydston et al. 2003b), then transfer directly into a new clan; they do not become nomads (Boydston et al. 2003b).

My first goal was to develop and validate age-estimation models for *C. crocuta* using data from known-age wild individuals. I addressed this goal by using multivariate analyses to reduce the number of independent variables that were subsequently used in the development of predictive regression models. My second goal was to test the hypothesis that secondary dispersal occurs in spotted hyenas and thus that not all interclan transfer is primary dispersal. To do this, I compared the ages of immigrating and emigrating males to determine what proportion might be secondary dispersers.

METHODS

I focused on one clan of spotted hyenas inhabiting the Talek region (1°40′S, 35°50′E) of the Masai Mara National Reserve, Kenya, in open grassland (Frank 1986a). From June 1988 through June 2001 this clan was observed for 23-31 days per month, except for April 1991 when hyenas were monitored for only 14 days. All individuals in the study clan could be recognized by their unique spot patterns. Spotted hyenas are strongly monomorphic in size and appearance (Hamilton et al. 1986), but observers were able to sex study animals by the dimorphic morphology of their erect phalluses (Frank et al. 1990). Ages of cubs were estimated to ±7 days when first observed based on pelage, size, and behavior (Holekamp & Smale 1998).

Hyenas were anesthetized with Telazol (W. A. Butler Co., Brighton, MI; 6.5 mg/kg) administered in a dart via a CO_2-powered rifle (Telinject Inc., Saugus, California). Most hyenas (76.8%, $n = 151$) were darted only once, but to accomplish other research objectives some hyenas were darted repeatedly. Cubs typically were darted when 8-12 months old ($n = 63$). Natal males also were darted when ≥24 months of age ($n = 14$), and natal females were darted again when believed to be pregnant ($n = 36$). As in prior work (e.g., Engh et al. 2002), immigrant males were considered resi

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resident if they stayed in the clan ≥6 months (*n* = 58), and most resident males (70.7%) were darted. Nonresident immigrant males (i.e., those that stayed in the clan ≤6 months) were excluded from all analyses. Eighteen resident immigrant males and 13 natal males were radio-collared. Those natal males known via radiotelemetry to establish residency in 1 clan (i.e., remained in a non-Talek clan for ≥6 months) before moving to a second clan were classed as secondary dispersers, as were radio-collared resident Talek immigrant males that subsequently established residency in another clan. Smale et al. (1997) used radiotelemetry to demonstrate that dispersing male hyenas usually take exploratory forays outside their natal home range before emigrating. Therefore, adult natal males without radio-collars were considered dispersers only if they had engaged in such excursions before their disappearance from the clan, if they were in good health when last seen, and if they were last seen in the Talek area when ≥24 months old. If these conditions were met, the estimated age of primary dispersal (*n* = 26) was the age of the male when he was last seen in the Talek territory before his first absence of ≥6 months.

Thirty-one morphological measurements (15 dental, 4 cranial, and 12 postcranial; Appendix A) were collected from hyenas immobilized (*n* = 228 dartings of 165 animals) and found dead (i.e., *n* = 33 necropsies, including 25 animals not darted). Whenever possible, skulls were collected from animals found dead. Incomplete observations were discarded, and measures from paired ipsilateral teeth (e.g., height of left and right p3) were averaged. The presence of deciduous teeth was noted, as was the sequence and timing of tooth eruption and replacement. Maternal rank at parturition was tested as a predictor of the age at initiation of tooth replacement among offspring in post hoc quantitative analyses. I chose maternal rank because it affects growth rates of cubs and ages at weaning (Hofer and East 1996; Holekamp et al. 1996) and thus indicates cub nutrition.

Darting data were sorted based on whether the permanent dentition was complete (i.e., subadult or adult). To avoid circularity and nonindependence during model validation, data were subdivided further into model-building and model-testing, or validation, data sets. Observations from animals without fully erupted permanent dentition were divided randomly into 2 samples of equal size for model construction (i.e., subadult model-building) and evaluation (i.e., subadult validation). Data from individuals with fully erupted permanent dentition were collected from

August 1989 through December 1999. If an animal was darted repeatedly, one darting was chosen at random to represent that individual in the adult model-building data set. The remaining adult records were combined with observations collected from January to June 2001 to form the adult validation data set. These data include a few nonindependent points (e.g., repeated dartings from 12 animals were included in both building and validation data sets), which had no appreciable effect on model performance and so were retained for model validation.

Multicollinearity among predictor variables and low ratios of sample size to predictor variables can produce spurious regressions (Neter et al. 1996). I conducted principal components analyses (PCA) on the correlation matrix of each model-building data set (Morrison 1990) to reduce iteratively the collinearity and dimensionality of the data while retaining the maximum amount of information. That is, after every PCA analysis I removed the independent variable with both the highest variable inflation factor and the strongest correlation to other variable(s), before again conducting PCA analysis. I repeated this process until the largest remaining variable inflation factor was less than 10 and the condition index was less than 100. These two measures indicate the degree of multicollinearity

in a data set (Neter et al. 1996), which may cause multiple linear regression models to perform poorly when applied to other data sets and can result in poor estimates of residual mean-square error. Multiple linear regression models were built with the remaining predictor variables (Appendix A). Various transformations (e.g., log, ln) of predictor and response data (i.e., age in months) were examined. No variable-selection method automatically will find the most appropriate predictor variables (Neter et al. 1996). Therefore I chose as final predictors those variables retained by forward, reverse, and stepwise variable selection procedures while minimizing the residual mean-square error, maximizing the coefficient of multiple determination (R^2), and minimizing the number of predictors.

I assessed a model by examining the accuracy (i.e., average discrepancy between estimated and known age) and precision (i.e., SD of estimation error) of the age estimates it produced from the validation data set. I deemed a model acceptably accurate if the average estimation error (i.e., discrepancy between estimated and known age) was ≤6 months, and acceptably precise if the SD of the error was ≤12 months. My initial goal in model building was to achieve these levels of accuracy and precision, but because none of the first multiple linear regression models met these criteria for estimating ages of animals with fully erupted permanent dentition, a second approach was used. I examined sexual dimorphism in dental morphology by combining all model-building and validation data from observations of complete permanent dentition, and then comparing known-age data from males and females for variables previously used in estimating the ages of *C. crocuta* (Kruuk 1972) and likely to vary with age (i.e., height and occlusal length of p3, height of upper and lower canines). I compared males and females with Mann-Whitney tests in age classes where sufficient samples existed for comparison. I set $\alpha = 0.05$ for all significance tests and used sequential Bonferroni adjustments to control for the effects of multiple comparisons (Rice 1989). Because hyenas are monomorphic (Hamilton et al. 1986) and known-age data for females were more extensive and variable than those for males, a model to estimate ages of males was built using data from females and tested with data from males. Correlations between estimated and known ages, and known ages and estimation error, were compared between models. Statistical analyses were conducted with statistical analysis software (SAS Inc. 1999).

I was able to create and test an age estimator based on the relationship Binder (1998) observed between height of

the 1st upper canine (C1) and age in young (≤40 months old) captive hyenas. I also assessed the estimator developed by van Jaarsveld et al. (1987) that predicted the height (mm) of p3 from the number of visible dentine lines in cross-sections of p3, with the latter as a proxy for age.

Using the best age-estimation model for adult males, I estimated age at darting of resident immigrant males and accounted for time elapsed since arrival in Talek (Holekamp & Smale 1998) to derive an estimated age at immigration. Estimated ages at immigration and known ages of emigration were compared with a 2-tailed chi-square test for homogeneity (Daniel 1990). Resident immigrant males were considered secondary dispersers into the Talek clan if their estimated age at arrival was > (maximum age of natal emigrants + maximum model overestimation error); otherwise they were considered primary dispersers. This produces a conservative estimate of the frequency of secondary dispersal. Values are presented as $\bar{x} \pm$ SD.

RESULTS

I gathered data from 191 complete known-age observations of 151 spotted hyenas (80 females, 71 male), ranging in age from 0.2 to 198 months (females aged $\bar{x}$ = 28.3 ± 46.1 months, males aged 17.1 ± 14.1 months). Permanent dentition of spotted hyenas includes i 3/3, c

1/1, p 4/3, m 0-1/1, total 32-34. Deciduous dentition consists of i 3/3, c 1/1, p 3/3, total 28. Substantial individual variation in timing of tooth eruption and replacement (Table 2.1) precluded a quantitative age estimator for cubs with only deciduous teeth. Deciduous incisors and canines had erupted by birth, and deciduous cheek teeth erupted by two months. Maternal rank at cub's birth, a proxy for nutritional state, was not associated significantly with the age at which the first deciduous teeth were replaced ($r = -0.074$, $n = 53$, $p = 0.596$). Eruption of the permanent dentition begins with the incisors (first, second, and third upper incisors and first, second, and third lower incisors in series), is followed by the cheek teeth (first, fourth, third, and second upper premolars and first lower molar, second, fourth, and third lower premolar in series), and ends with the canines. The last deciduous teeth were replaced by 13-18 months of age. I found no differences between males and females of the same age in height (mm) or occlusal length (mm) of p3, or height (mm) of upper or lower canines (all $p > 0.05$; Figure 2.1). Therefore data from both sexes were pooled.

Table 2.1—Tooth eruption in 144 known-age, free-living *Crocuta crocuta* (from 119 dartings, 25 necropsies).

Dentition type	Age range (months)	*n*
No adult teeth	0.5-7.5	25
Only adult I1 present	6.5-9.0	16
Adult I1 and I2 present	7.8-12.0	43
Some adult cheek teeth present	10.0-15.0	38
All adult teeth present	13.0-18.0	22

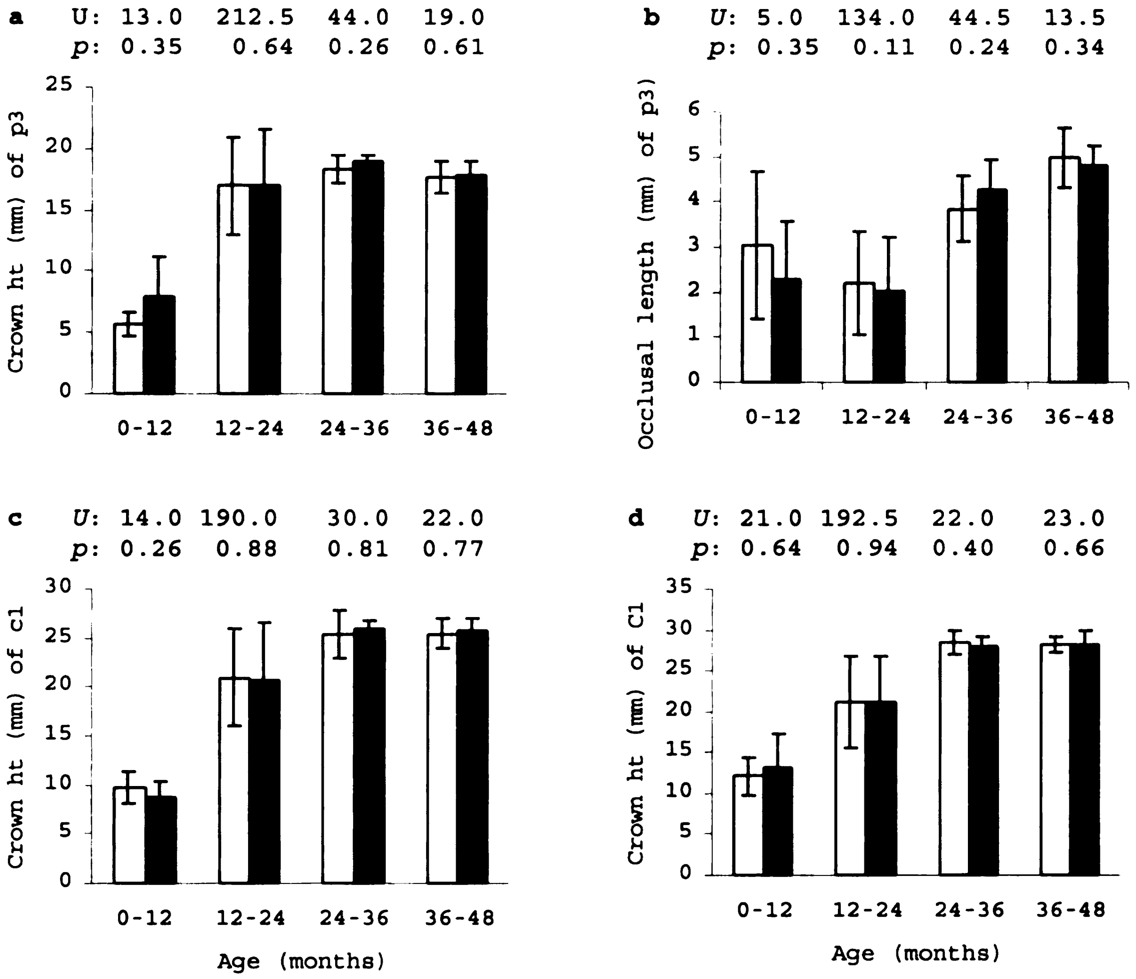

Figure 2.1—Tooth measurements of known-age free-living *Crocuta crocuta*. White bars are values for males, gray bars for females, shown as $\bar{x} \pm SD$. a) Height of p3 (p3), b) occlusal surface length of p3 (OCCp3), c) height of c1 (c1), and d) height of C1 (C1). Variables are defined in Appendix A. Sample sizes are noted on bars. Two males were darted when >48 months old (i.e., 60 months, 88.5 months); measurements from these males lie within the range of values from females of similar age.

The subadult model-building data set included 19 (13 female, 6 male) known-age observations (12.7 ± 1.8 months) and was validated with 19 (7 female, 12 male) known-age observations (12.7 ± 1.8 months). Selection procedures for variables in multiple linear regression suggested that two variables be used: height (mm) of the first lower canine (c1) and height (mm) of the first upper canine (C1; Appendix A). The best model for animals with incomplete permanent dentition ($F = 32.48$, $d.f. = 2, 16$, $p < 0.0001$, $R^2 = 0.805$) predicted age as

$$\mathrm{Log}_{10}(\text{age in months}) = 0.647 + 0.158[\log_{10}(\mathrm{C1})] + 0.238[\log_{10}(\mathrm{c1})] \quad (1)$$

I found a positive correlation between known ages and estimates from this model (Figure 2.2). I also found a negative correlation between known ages and this model's estimation errors (Table 2.2), as is often seen in models based on tooth wear. Nevertheless, this model performs acceptably.

The adult model-building data set of 60 (38 female, 22 male) known-age observations (56.6 ± 40.7 months) was used to construct mixed-sex models to produce age estimates for

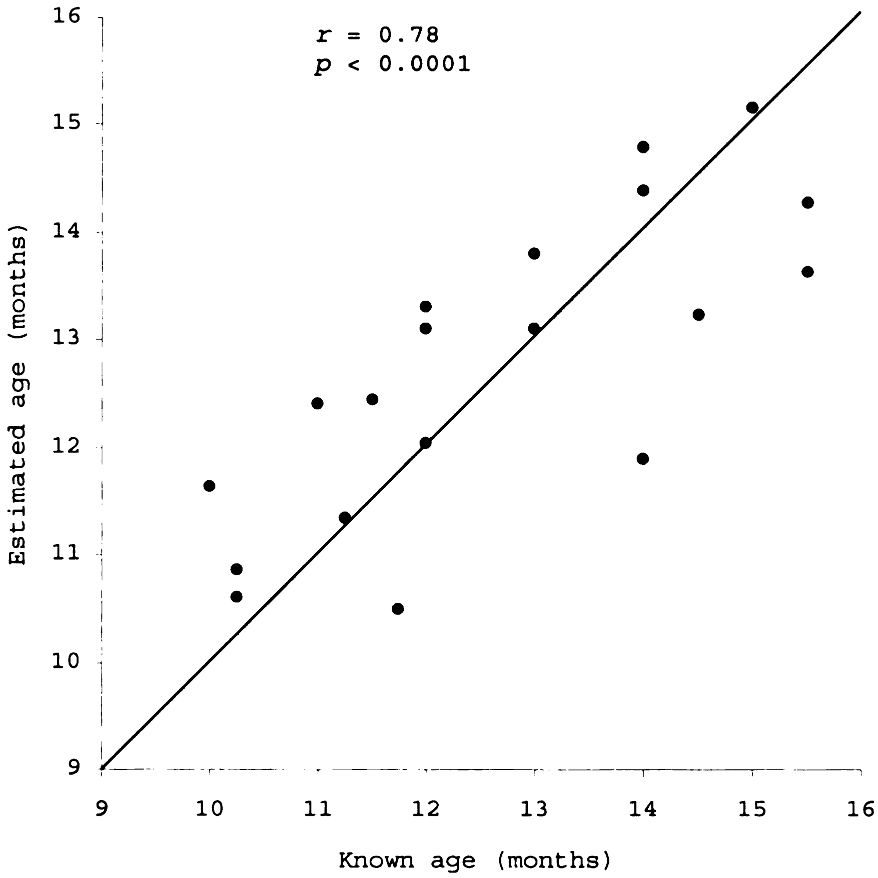

Figure 2.2—Association of known and estimated ages ($n = 19$) of male and female *Crocuta crocuta* with erupting permanent dentition from regression (equation 1) of $\log_{10}$(age in months) on $\log_{10}$(C1) and $\log_{10}$(c1). Solid line indicates theoretically perfect association.

Table 2.2—Validation of models to estimate age in free-living *Crocuta crocuta* from tooth measurements, including number of observations used in model derivation (n_d) and validation (n_v), with the residual mean-square error and coefficient of multiple determination (R^2) from each equation. A negative error indicates that the regression underestimated the known age. See text for equations describing models.

Model estimating age	n_d	n_v	Residual mean square error	R^2	Estimation error (months) $\bar{x} \pm SD$	Estimation error (months) *Range*	Association of age and error r	Association of age and error p
(1)	19	19	0.911	0.805	0.11 ± 1.13	-2.11 to 1.62	-0.616	0.005
(2)	60	27	15.762	0.855	1.07 ± 22.63	-63.51 to 55.98	-0.356	0.069
(3)	60	27	0.104	0.877	-2.65 ± 26.56	-82.34 to 65.31	-0.126	0.530
(4)	56	28	0.085	0.920	-0.54 ± 4.94	-12.96 to 10.91	-0.411	0.029

either females or males, which were tested with the adult validation data set (18 female, 9 male; 58.8 ± 39.5 months). Concordance among selection methods for variables in multiple linear regression suggested that 2 predictor variables be used: occlusal length (mm) of p3 (OCCp3; Appendix A), and height (mm) of c1. The best model for mixed-sexed observations ($F = 168.58$, $d.f. = 2, 57$, $p < 0.0001$, $R^2 = 0.855$) predicted age as

$$\text{Age in months} = 34.281 + 15.423(\text{OCCp3}) - 2.473(\text{c1}) \quad (2)$$

However, spotted hyenas often break c1 (Van Valkenburgh 1988). If c1 was excluded from the analysis, the resulting models were less accurate and precise. The best of these models ($F = 412.27$, $d.f. = 1, 58$, $p < 0.0001$, $R^2 = 0.877$) was

$$\text{Log}_{10}(\text{age in months}) = 1.004 + 0.116(\text{OCCp3}) \quad (3)$$

Both mixed-sex models have acceptable accuracy (Table 2.2) and produce estimates that are correlated positively with known ages (Figure 2.3). The association between estimation error and known age was weak and nonsignificant for both models (Table 2.2). The only trend in error was

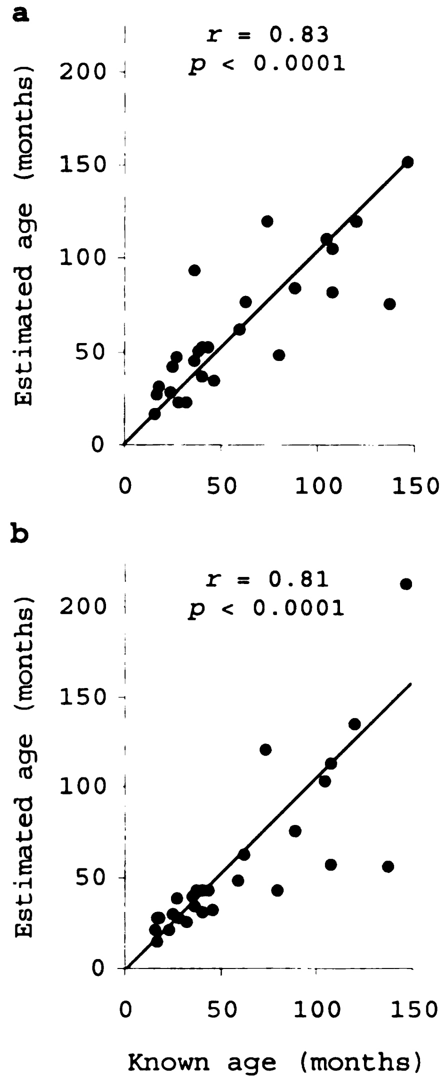

Figure 2.3—Association of known and estimated ages (n = 27) of male and female *Crocuta crocuta* a) from regression (equation 2) of age (in months) on p3 and c1, and b) from regression (equation 3) of $\log_{10}$(age in months) on p3. Solid line indicates theoretically perfect association.

that both models were less precise for animals ≥72 months old (Figure 2.3). No model built with data from males to predict ages of females performed as well as models (2) or (3), but this was expected because fewer males were sampled at many ages.

The best model ($F = 217.59$, $d.f. = 3, 56$, $p < 0.0001$, $R^2 = 0.92$) for estimating ages of adult males ($n = 28$, 30.4 ± 15.4 months) was built with data on known-age females ($n = 56$, 73.8 ± 41.5 months) and estimated age of males as

$$\text{Log}_{10}(\text{age in months}) = 1.237 - 0.070(\text{OCCp3}) + 0.040(\text{OCCp3})^2 - 0.002(\text{OCCp3})^3 \qquad (4)$$

This model produced a positive correlation between estimated and known ages (Figure 2.4), and a negative correlation between known ages and estimation errors (Table 2.2). The model met my overall accuracy and precision criteria (Table 2.2) and did not decline in precision among the oldest males, which are much younger than the oldest females (Figure 2.4).

Model (4) was used to estimate ages at arrival of resident Talek immigrants (45.4 ± 21.1 months). These estimated ages at immigration were different (Figure 2.5) from known ages at emigration from Talek ($n = 40$, 42.1 ±

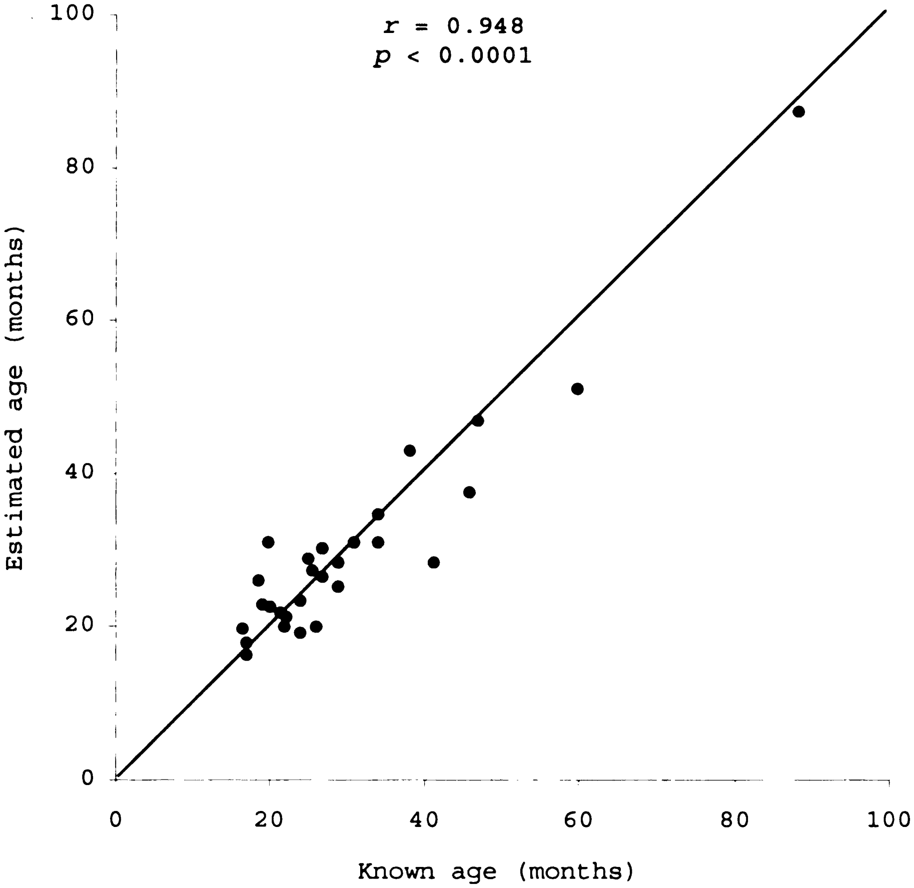

Figure 2.4—Association of known and estimated ages (n = 28) of male *Crocuta crocuta* from regression (equation 4) of $\log_{10}$(age in months) on OCCp3. Solid line indicates theoretically perfect association.

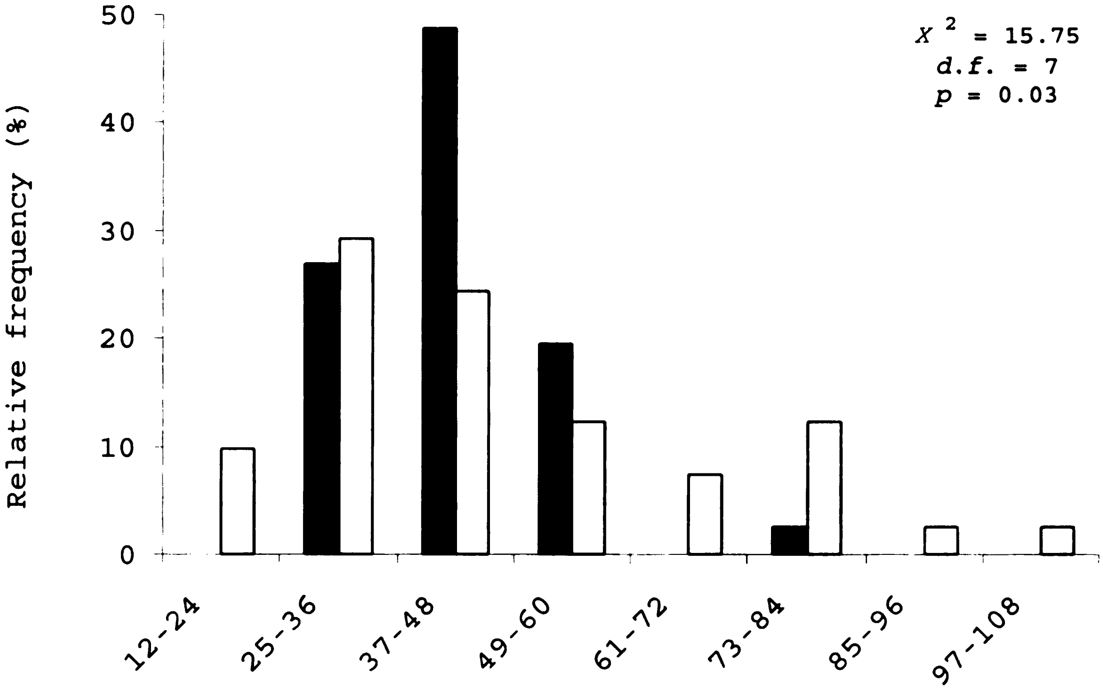

Figure 2.5—Age distributions of Talek emigrants (black bars, *n* = 40) and resident immigrant (white bars, *n* = 41) male *Crocuta crocuta* at dispersal. Resident immigrants were those that remained in the Talek clan for ≥6 months.

10.5 months). Because the oldest (i.e., 75.9 months) disperser from Talek was more than one *SD* older (i.e., 15.3 months) than the next oldest disperser, and missed my absence cutoff by only 1.1% (i.e., 2 days < 6 months), I consider his dispersal age an outlier. Based on the other observed ages at dispersal and model estimation error, resident immigrants who were estimated to be ≤70.1 months old at arrival were considered primary dispersers. Most resident immigrants appeared to be primary dispersers (*n* = 34, 37.6 ± 12.4 months), but 17% (*n* = 7, 83.0 ± 11.9 months) were evidently engaging in secondary dispersal when they joined the Talek clan. Fates of 20 males were known via radiotelemetry, which revealed that secondary dispersal occurred among a substantial minority (40%) of both natal (2 of 9) and resident immigrant (6 of 11) males. Small sample sizes and methodological differences precluded comparison of the rates of secondary dispersal between natal and resident immigrant males.

DISCUSSION

The sequence of tooth replacement observed here matched that seen in spotted hyenas by Slaughter et al. (1974), including variability in replacement of P2 and p2. As I expected based on earlier studies (Schneider 1926; Pournelle 1965; Frank et al. 1991), the deciduous incisors and canines were erupted at birth. Pournelle (1965) noted the eruption of deciduous cheek teeth at 31 days of age, consistent with my observation of the eruption of those teeth by 2 months of age. Binder and Van Valkenburgh (2000) noted that adult dentition of captive spotted hyenas might be fully erupted at younger ages (i.e., 12-14 months) than in wild conspecifics. They observed ages that agree with those of Kruuk (1972) from wild spotted hyenas, but which are younger than those I or Mills (1990) observed. The faster eruption observed in captive animals might result from improved nutrition. The softer, yet more nutritious diet of young (i.e., ≤40 months old) captive spotted hyenas (Berger et al. 1992) also might have caused the regression derived from Binder (1998) to underestimate the ages of young free-ranging hyenas. This suggests that variation in nutritional status among wild spotted hyenas may influence the rate of tooth eruption and replacement and explain some of the estimation error of my model for animals with subadult dentition. I could not measure directly the nutritional status of wild hyena cubs, but a surrogate measure of this, maternal rank, was not associated with the age at which tooth replacement was initiated. Nonetheless, maternal rank may play a greater role in dental development in cubs when females are under greater nutritional stress than in my study. Genetic variation may explain some of the estimation error of my models, but the data to assess the impact of genetic variation are not yet available from a sufficiently large multi-generational sample.

I tested an estimator derived from Binder (1998) with data from free-ranging animals similar in age to those she studied ($n = 12$, 26.7 ± 8.2 months). This model produced estimates, from the height of permanent C1, not correlated with known ages ($r = 0.276$, $p = 0.386$), and it consistently underestimated known ages (error = -23.0 ± 7.9 months). The mixed-sex model of van Jaarsveld et al. (1987) produced estimates that were positively correlated with known ages ($r = 0.756$, $p < 0.0001$), but it overestimated the age of all but 1 individual ($n = 27$, error = 73.2 ± 34.8 months). This model yielded no association between estimation error and known age ($r = 0.02$, $p = 0.92$).

van Jaarsveld et al. (1988) described strong relationships between ages estimated from dental annuli and morphometric data (e.g., shoulder height, body weight, total body length) in South African spotted hyenas, but my PCA analyses indicated that these variables should be discarded from the data set prior to the final regression analyses and model selection. Rather, age of Talek hyenas was best estimated by dental measurements. Spotted hyenas crush and consume large amounts of bone (Kruuk 1972; Mills 1990) using p3 (Ewer 1954; Werdelin & Solounias 1991; Biknevicius 1996; Van Valkenburgh 1996; Binder & Van Valkenburgh 2000), so it is not surprising that wear of this tooth reflects age. Another advantage to using p3 in an age estimator for spotted hyenas is that breakage of this tooth is less common than that of other teeth (Van Valkenburgh 1988). I recommend that researchers calculate the mean anterior-posterior occlusal length (mm) of both right and left p3 to reduce estimation error due to breakage and asymmetrical tooth wear. The latter appears common in wild *C. crocuta* (Lindeque & Skinner 1984).

Overestimation of ages with the equation of van Jaarsveld et al. (1987) is consistent with observing that the maximum estimated age for spotted hyenas in that study was 7 years greater than the maximum known age among Talek hyenas (i.e., about 24 years versus 17 years). This overestimation might be due to irregular formation of dentine lines or to differences in tooth wear between spotted hyenas in Kenya and South Africa. Such interpopulation variation has been observed in roe deer (*Capreolus capreolus*—Hewison et al. 1999) and Spanish ibex (*Capra pyrenaica*—Fandos et al. 1993) but has not been seen in carnivores (Harris 1978; Smuts et al. 1978; Gipson et al. 2000), and data on diet and tooth wear are not yet available from multiple populations of spotted hyenas. Because of the possibility for variation in tooth wear among populations, my models should be validated with known-age data whenever possible.

Spinage (1973) predicted that tooth wear would overestimate the ages of young animals and underestimate the ages of old animals. This has been observed in roe deer (Hewison et al. 1999) and with my best estimator of male age (i.e., equation 4). Nonetheless, I find the error of my model acceptable, and this is the model I recommend for estimating the ages of adult male *Crocuta*. Previous researchers experienced difficulty in estimating the ages of older gray wolves (*Canis lupus*—Landon et al. 1998), red deer (*Cervus elaphus*—Brown & Chapman 1991), and fallow deer (*Dama dama*—Brown & Chapman 1990), and my best estimator of female ages (i.e., equation 2) also had decreased precision among older individuals. This decreased precision occurs after an age (i.e., 72 months) older than most (i.e., 76%) of the animals whose measurements were used to build and test the mixed-sex models. Therefore I conclude that my model provides realistic estimates across most of the lifespan of wild *C. crocuta*. I recommend this equation to estimate the ages of female spotted hyenas or hyenas of unknown sex.

Sex affects age estimation in white-tailed deer (*Odocoileus virginianus*—Van Deelen et al. 2000). Because I found no sexual dimorphism in those variables that best reflected and predicted age, and because my best estimator of male age was built with data from females, I agree with those (Hamilton et al. 1986; van Jaarsveld et al. 1988) who have suggested that sexual dimorphism among spotted hyenas is very slight. Individual variation in tooth wear has been observed in roe deer (Hewison et al. 1999) and red fox (Harris 1978) and linked to variation in enamel mineralization within red deer (*Cervus elaphus*) populations (Kierdorf & Becher 1997). I propose that individual variation is more important than sexual dimorphism in shaping tooth wear among spotted hyenas. Social rank may influence diet composition and cumulative differences in tooth wear, but I cannot address this with my current data.

Natal Talek males disperse at approximately the same ages as males in the adjacent Serengeti ecosystem (East & Hofer 2001). Talek males do not become nomadic (Boydston et al. 2003b), so older immigrants in this area must be secondary dispersers. Secondary dispersal occurs frequently among male *C. crocuta* in my study area, so rank of immigrant males in Talek is not as closely linked to age as has been suggested to be the case in the Serengeti (East and Hofer 1991), where secondary dispersal appears to occur rarely (Hofer et al. 1993). Secondary dispersal in the Serengeti may be constrained by more limited opportunities for dispersal (Boydston et al. 2003b). Genetic analyses have shown that primary dispersal by males is a near-universal prerequisite for reproductive success in Talek (Engh et al. 2002), so males may also undergo secondary dispersal to improve their reproductive success. However, I do not know whether secondary dispersers accrue increased reproductive benefits in their new clans.

CHAPTER THREE

ROLE-REVERSED NEPOTISM AMONG CUBS AND SIRES IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

INTRODUCTION

Nepotism is favoritism based on kinship; such favoritism may allow an individual to achieve higher inclusive fitness through indirect selection than an individual that does not bias its behavior according to kinship (Hamilton 1964). Kin recognition (Holmes & Sherman 1982), the process by which kin are discriminated from unrelated conspecifics, is not sufficient for the expression of nepotism (Waldman et al. 1988; Sherman et al. 1997; Shettleworth 1998; Tang-Martinez 2001; Silk 2002). In fact, individuals that recognize one another as kin may be nepotistic in some circumstances but not others (e.g., Mateo & Johnston 2000).

Nepotism occurs most commonly between parents and immature offspring, yet there is a "fundamental asymmetry" (Hamilton 1964, pg. 2) in regard to this nepotism. That is, parents are more physically capable and experienced than their progeny, so the relative costs of most behaviors are lower for a parent than for its offspring. Parents in many species recognize their offspring and bias their behavior accordingly in parental nepotism (e.g., Insley et al. 2003) to increase the direct fitness of both the offspring and the discriminating parent (e.g., Lynn & Wingfield 2003); other parents either cannot or do not do so (e.g., Pillay 2000). Nepotism by offspring towards their parents occurs most often as assistance during agonistic interactions (Harper 1981) or as helping during cooperative breeding (e.g., Komdeur 2003), but I am unaware of any cases where filial nepotism directed by progeny towards their parents is greater than parental nepotism. Filial nepotism is undoubtedly usually constrained by the small size and inexperience of offspring, especially among mammals, but this may not be true among spotted hyenas (*Crocuta crocuta*).

Spotted hyenas are gregarious carnivores that live in social groups called clans (Kruuk 1972). Clans in east Africa typically contain multiple matrilines of females and their offspring, and multiple adult males that have joined the clan from elsewhere. Females of multiple generations from multiple matrilines rear their cubs together at a communal den (Kruuk 1972; East et al. 1989; Hofer & East 1993a). Each clan is rigidly structured by a stable linear dominance hierarchy in which an individual's social rank determines its priority of access to food (Kruuk 1972; Tilson & Hamilton 1984; Frank 1986b). A female's social rank determines the social rank of her offspring within their natal clan; maternally related hyenas therefore hold adjacent social ranks (Holekamp & Smale 1991; Engh et al. 2000). Females rarely disperse, but virtually all males disperse from their natal clans after puberty (Frank 1986b; Hofer & East 1993b; Smale et al. 1997; East & Hofer 2001). Each new immigrant assumes the lowest rank in the clan's hierarchy (Smale et al. 1993; Pusey & Packer 1997; Smale et al. 1997; Holekamp & Smale 1998); his rank improves only through attrition of males that outrank him (Pusey & Packer 1997; Smale et al. 1997). Intrasexual dominance does not determine which males sire cubs (Engh et al. 2002; East et al. 2003), but a male's dispersal status has strong influence: immigrant males sire nearly all cubs even though they rank below all adult natal males that have not yet dispersed (Engh et al. 2002). In fact, all immigrant males remain subordinate to all natal animals including their own cubs, regardless of their relative body size (Smale et al. 1993; Smale et al. 1997; Holekamp & Smale 1998; East & Hofer 2001). Thus, the rank advantage of spotted hyena cubs over their sires creates an unusual opportunity for filial nepotism to be effective and influential.

Spotted hyenas exhibit nepotism among maternal kin: with rare exceptions (Knight et al. 1992), females nurse, provision, and groom only their own cubs (Kruuk 1972; Mills 1985; Holekamp & Smale 1990; Pusey & Packer 1994). Maternal kin associate more tightly, are more affiliative, and hunt together more than with others (Mills 1985; East et al. 1993; Holekamp et al. 1997a). In addition, females recognize and respond to the vocalizations of close maternal kin (Holekamp et al. 1999a). However, these studies were conducted without knowledge of paternal kinship. Wahaj et al. (2003) have recently shown that nepotism occurs between both maternal and paternal siblings, but it is unknown whether or not nepotism also occurs between offspring and their sires.

My first goal was to test the hypothesis that spotted hyenas exhibit filial nepotism towards their sires, to whom they are socially dominant. My second goal was to test the reciprocal hypothesis, that immigrant male spotted hyenas exhibit paternal nepotism towards their offspring.

METHODS

Study site and subjects

I studied spotted hyenas of the Talek clan in the Masai Mara National Reserve, Kenya (Frank 1986a). I considered all animals born in the clan as natal animals, and classified with them as residents all immigrant males remaining in the clan for at least six months; immigrant males that remained in the clan for less than six months were excluded from this study. Immigrant males rarely sire cubs during their first six months of residency in this clan (Engh et al. 2002). The Talek clan contains 60-80 resident hyenas and defends an area of approximately 65 km^2 (Boydston et al. 2001). All members of the Talek clan were identified by their unique spot patterns, and sexed based on the dimorphic glans morphology of the erect phallus (Frank et al. 1990). Cub birth dates were estimated to ± 7 days (Holekamp & Smale 1993; Holekamp et al. 1996). I assigned social rank to Talek hyenas based on the appeasement behavior they displayed during dyadic agonistic encounters (Martin & Bateson 1988; Engh et al. 2002).

Assigning paternity

I used molecular genetic data to determine paternity and confirm maternity assigned from nursing, as in Engh et al. (2002). DNA for paternity analysis came from the blood of darted hyenas and tissue from known animals found dead. Darted hyenas were anesthetized with Telazol (W. A. Butler Co., Brighton, Michigan, U.S.A; 6.5 mg/kg) administered in a plastic dart via a CO_2-powered rifle (Telinject Inc., Saugus, California, U.S.A.). Cubs were typically darted when 8-12 months old, and nearly all (92%) immigrant males (n = 109) were also darted (Engh et al. 2002). DNA was extracted in the field from blood samples with a Puregene kit (Gentra Systems Inc., Minneapolis, Minnesota, U.S.A.) and stored in liquid nitrogen (Engh et al. 2002). Tissue was also stored in liquid nitrogen until DNA could be extracted with either standard phenol-chloroform extraction techniques or Qiagen kits (Qiagen Inc., Valencia, California, U.S.A.) in the laboratory. I amplified 11 autosomal microsatellite loci and one X-linked microsatellite locus using conditions described previously (Libants *et al.* 2000, Engh 2002). All adult males residing in the clan at conception of a particular litter were considered as possible sires, except for close maternal kin of the mother (r > 0.125), which were excluded *a priori* to avoid assigning spurious paternities to close male kin. I concluded that a male was the actual sire of a cub when he was assigned paternity through simple exclusion or when paternity was assigned with 95% confidence by the software program Cervus (Marshall et al. 1998). I assigned 64 paternities to 15 different sires.

Collection of behavioral data

The Talek clan was observed on at least 23 d/mo from June 1988 through October 1998, except during April 1991 when hyenas were monitored for only 14 days. Observations were made from parked vehicles, primarily from 05:00 to

09:00 and from 17:00 to 20:00. An observation session lasted from five minutes to several hours, beginning when one or more hyenas was found separated from others by at least 200 m, and ending when observers left the group. All occurrences of aggressive behavior during observation sessions were recorded as critical incidents (Altmann 1974). In addition, data on affiliation were collected during focal animal surveys (Altmann 1974) of cubs or adult immigrant males when both were present. Mean duration of these focal animal observations was $\bar{x}$ = 27.6 min ± 1.8 SE.

I inquired whether nepotism influenced affiliative behavior between offspring and sires, relative to that observed between unrelated pairs of cubs and immigrant males. To determine whether cubs exhibited more affiliation towards their sires than to unrelated adult immigrant males, I chose as control males the immigrants next lowest in rank to the actual sires. To determine whether sires exhibited more affiliation towards their own cubs than to unrelated cubs, I chose as controls cubs with the nearest possible dates of birth to the focal cubs but with different parents. I considered affiliation to consist of non-aggressive approaches and greetings because both behaviors have been shown to serve affiliative functions in spotted hyenas (East et al. 1993; Wahaj et al. 2001). From focal animal surveys I calculated rates of approaches to within 1 m, and rates at which greeting behaviors occurred. A greeting occurs when one animal lifts its hind leg, presenting its genitals to a second animal, which often responds by sniffing the first animal's genitals, lifting its own hind leg, and presenting its own genitals (East et al. 1993). Thus, I was able to note which individual initiated greetings by noting which individual was first to raise its leg. In addition, I used focal animal data to calculate the rates at which cubs and adult immigrant males played together, because rates of play are known to vary among kin classes and reveal kin discrimination among siblings (Wahaj et al. 2003). Observers recognized play behavior by an animal's exaggerated movements and the absence of aggressive postures or vocalizations. I also used focal animal data to calculate the rates at which cubs tolerated feeding by adult immigrant males at carcasses and kills. Because cub rank confers priority of access to food over all immigrants, tolerance was defined as the absence of attempts by a cub to exclude an immigrant male from a food source. Tolerance at a food source represents one way in which a cub might directly improve its sire's fitness.

I compared the rates at which cubs directed each behavior towards their sires with the rates at which these same cubs directed that behavior towards control males. I also compared the rates at which sires directed each behavior towards their own cubs with the rates at which these same sires directed that behavior towards control cubs. I predicted that greetings and approaches would be directed at higher rates to kin than to nonkin. To avoid confounding the effects of reproductive behaviors with those of nepotism, I used focal animal data only from cubs younger than 24 months, the minimum age of puberty for male and female spotted hyenas (Holekamp et al. 1996; Smale et al. 1997). Furthermore, I used data from paired cubs and adult males only if they were observed together for at least 60 focal animal min.

In addition to altering rates of affiliation, nepotism might alter rates and intensities of aggressive behaviors between cubs and sires, as it does between paternal siblings (Wahaj et al. 2003). Because of their low social status, immigrants never direct aggression towards cubs (Smale et al. 1993; Holekamp & Smale 1998). However, if cubs recognize their sires, they should direct lower rates or intensities of aggression towards sires than to control males if they reap a net benefit by doing so. Therefore, I compared the hourly rates of aggression initiated by cubs against their sires to the hourly rates of aggression initiated by those same cubs against unrelated immigrant males. Using the same animals as those used in analyses of affiliation, I calculated hourly rates of low and high intensity aggressions from critical incident data (Altmann 1974). Low intensity aggressions were head waves; high intensity aggressions included displacements, lunges, aggressive posturing (i.e., ears cocked forward while the tail was bristled and raised), standing over an opponent, pushing, chasing, and biting.

Kinship affects the degree to which natal clan members associate with one another (Holekamp et al. 1997a; Wahaj et al. 2003); if they can recognize each other, cubs might be expected to associate more closely with their sires than with unrelated immigrant males, and sires might associate more closely with their offspring than with unrelated cubs. Because association between spotted hyenas is influenced by mate choice (Szykman et al. 2001) and influences male mating success (Szykman 2001 but see East et al. 2003), I selected control cubs of the same sex as a male's actual cubs. I calculated the twice-weight association index (AI) of Cairns and Schwager (1987) for cubs and their sires, cubs and control males, and sires and control cubs. To provide a frame of reference by which to gauge the strength of associations between cubs and males, I also calculated

AI between these cubs and their mothers. The AI between animals A and B was calculated by dividing the number of observation sessions in which both A and B were present by the sum of that number plus the number of sessions in which either A or B was observed without the other being present (Cairns & Schwager 1987). I counted an animal as present if it was observed for part or all of an observation session. To avoid the potentially confounding impact of the mother's presence on the AI of cubs and males, I excluded all observation sessions at which a cub's mother was present from the calculations of AI among cubs and males. Young hyenas spend several months at the communal den where they are visited by their mothers and other clan members, most of whom are dominant to the cubs' sires. To avoid the confounding effects of the communal den, AIs were only calculated for the period after cubs became independent of the communal den (Boydston et al. 2003a). Time intervals for AI calculations began when both focal and control cubs were independent of the communal den; intervals ended with the first disappearance of any dyad member. The time periods for AI calculation were thus unique to each set of related and control animals. To avoid spuriously low AIs, I included only comparisons in which this period of overlap was at least 1 month long.

Statistical analyses

I compared rates of affiliative and aggressive behaviors with Wilcoxon matched-pairs signed-rank tests. Because my hypotheses generated directional predictions regarding comparisons of rates and intensities of behaviors between dyad types, I conducted one-tailed tests. I compared the AI exhibited by male cubs with sires and control males to the AI of female cubs with sires and control males, using two-tailed Mann-Whitney tests; I saw no statistically significant differences between the sexes so I pooled the data and compared AIs between cubs and sires to AIs between cubs and unrelated immigrant males with one-tailed Wilcoxon matched-pairs signed-rank tests. Some sires (n = 7) had both male and female cubs in the data set; I compared the average AI of these sires with their sons and daughters through Wilcoxon matched-pairs signed-rank tests to determine whether cub sex had an influence on sire-cub AI. After I saw a statistically significant impact of cub sex on sire-cub AI, I compared the sex-specific AIs of sires and cubs with Mann-Whitney tests. The unit of analysis for all comparisons was the individual, and in all analyses I set p 0.05. All statistical analyses were conducted in Systat 8.0 (SPSS Inc. 1998), and data are reported as $\bar{x}$ ± SE.

RESULTS

Twelve cub-sire pairs (5 sons, 7 daughters, 7 sires) were observed for at least 60 min during focal animal surveys conducted over 22.15 ± 4.38 months. I gathered data on affiliative and aggressive behaviors from these focal animal surveys (n = 572, 244.82 h). AIs were calculated for 46 cubs with their mothers, their sires, and unrelated immigrant males across 12.94 ± 1.8 months, after the cubs had become independent of the clan's communal den.

Kinship did not significantly affect rates of affiliation between cubs and immigrant males. Although cubs only rarely initiated greetings with their sires (0.095 ± 0.083 greetings/h), they never did so with unrelated immigrant males; this difference was not statistically significant ($Z = 1.342$, $p = 0.09$). Sires initiated greetings with their cubs more than three times as frequently (0.113 ± 0.083 greetings/h) as with unrelated cubs (0.036 ± 0.023 greetings/h), but this difference was also not statistically significant ($Z = -0.944$, $p = 0.173$). Cubs approached their sires (0.345 ± 0.113 approaches/h) and unrelated immigrant males (0.454 ± 0.266) at similar rates ($Z = -0.28$, $p = 0.39$). Although sires approached their cubs three times as frequently (0.272 ± 0.132 approaches/h) as they approached unrelated cubs (0.095 ± 0.057 approaches/h), this difference was not statistically significant (Z = -1.153, p = 0.125). Feeding tolerance by a cub towards any immigrant male was seen only once during any focal animal survey, and this involved a cub and its sire. Cubs and immigrant males never played together. Although there was no statistical support for nepotism during affiliative behaviors, trends in the data suggested that cubs and sires recognize each other as such.

Kinship affected the intensity with which cubs directed aggression towards immigrant males. During 1,747.05 h of critical incident sampling, cubs exhibited low intensity aggression towards their sires and unrelated immigrant males at similar rates (Z = 0.652, p = 0.258; Figure 3.1). However, cubs directed high intensity aggression towards their sires less often than towards unrelated immigrant males (Z = 1.992, p = 0.023; Figure 3.1). Thus, cubs exhibited nepotism by reducing the intensity of aggression against their sires.

Kinship significantly influenced the degree of association between adult males and offspring (n = 46) after cubs became independent of the communal den at an average age of 8.02 ± 0.264 mo. Cub sex (n = 22 females, n = 24 males) had no impact on AIs with either sires (0.043 ±

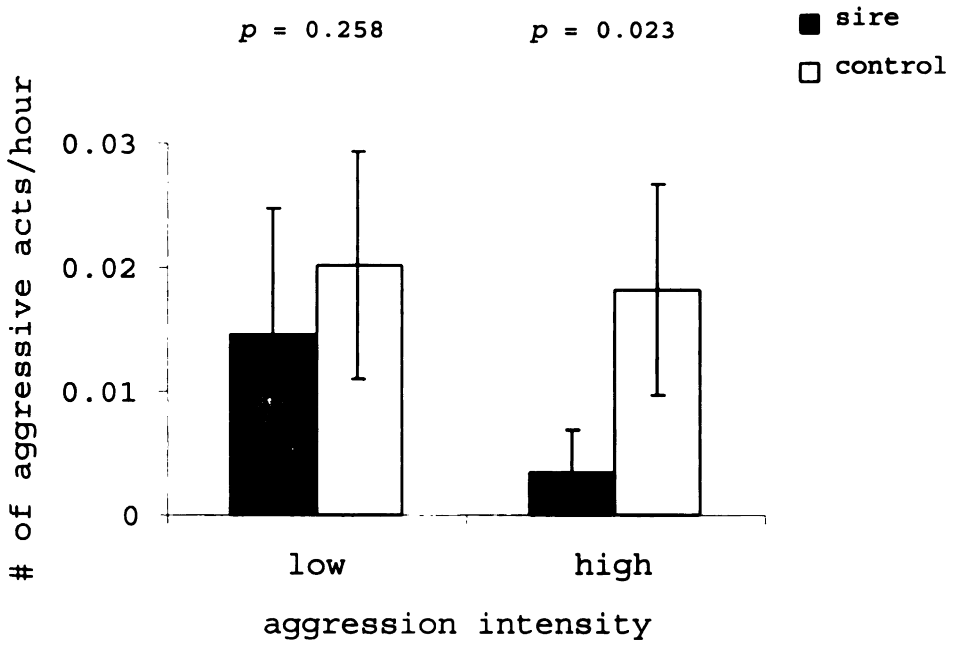

Figure 3.1—Hourly rates ($\bar{x}$ ± SE) at which spotted hyena cubs (n = 12) directed aggressive acts at their sires and at unrelated immigrant males (controls). Aggressive behaviors are described in the text.

0.009 females, 0.046 ± 0.004 males; $U = 212.0$, $p = 0.253$) or unrelated immigrant males (0.035 ± 0.005 females, 0.036 ± 0.005 males; $U = 266.0$, $p = 0.965$). Den-independent cubs ($n = 46$) associated more with their mothers (0.212 ± 0.017) than with either their sires (0.045 ± 0.005; $Z = -5.861$, $p < 0.001$) or unrelated immigrant males ($Z = -5.894$, $p < 0.001$). In addition, the average AI between an offspring and its sire was significantly higher ($Z = -1.869$, $p = 0.031$) than the AI between that cub and an unrelated immigrant male (Figure 3.2a). Seven immigrant males sired both daughters ($n = 3.14 \pm 1.06$ daughters/sire) and sons ($n = 2.57 \pm 0.69$ sons/sire) with whom they coexisted in the clan for at least one month after the cub became independent of the communal den. Offspring sex influenced the average AI between these sires and their daughters and sons ($Z = -2.366$, $p = 0.018$; Figures 3.2b, 3.2c), but not the average AI between these sires and unrelated juveniles ($Z = -0.338$, $p = 0.735$; Figures 3.2b, 3.2c). Average AI was greater between sires and their daughters than between sires and unrelated young females ($Z = -2.711$, $p = 0.004$; Figure 3.2b), but there was no difference among the average AI between sires and their sons and between sires and unrelated young males ($Z = -1.2$, $p = 0.115$; Figure 3.2c).

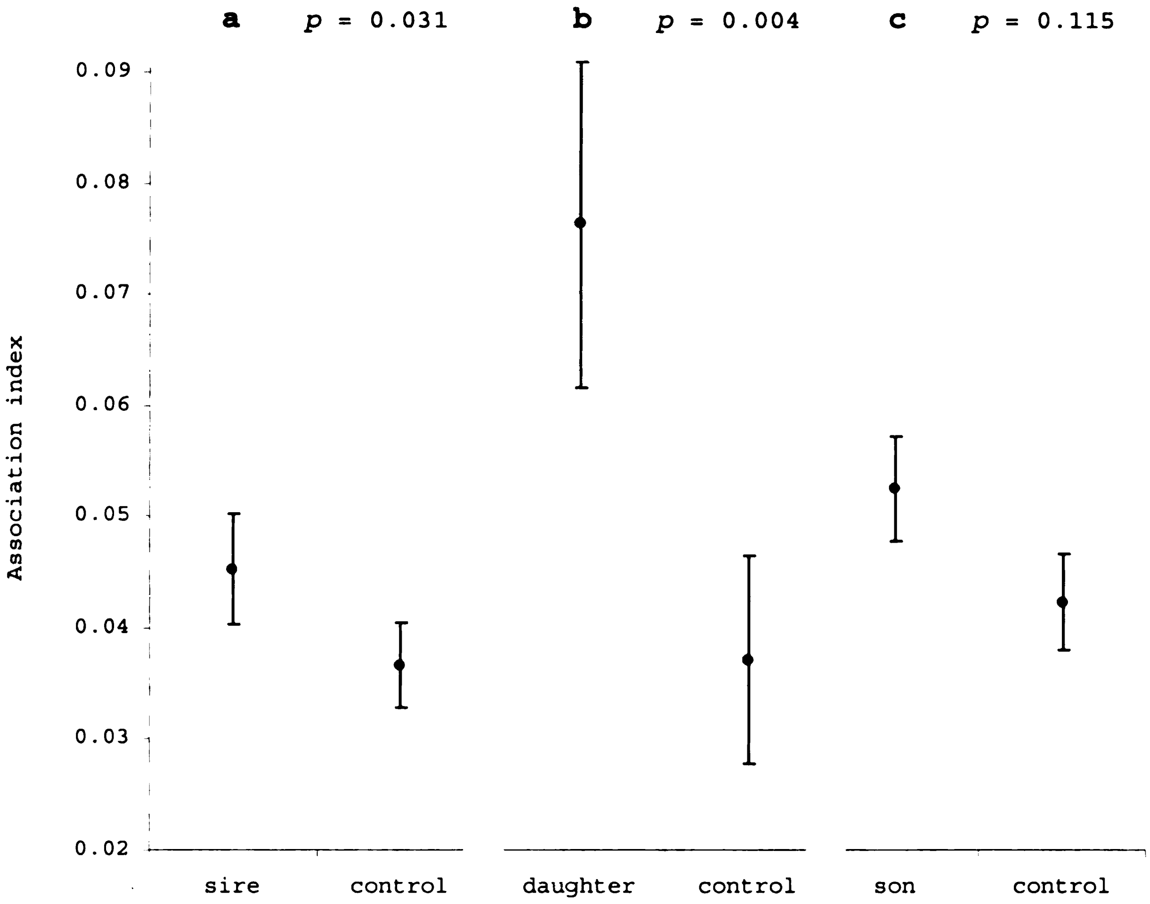

Figure 3.2—Association indices ($\bar{x}$ ± SE) between cubs (n = 46) and immigrant males (a), between sires (n = 7) and their daughters (n = 22) (b), and between these same sires and their sons (n = 24) (c). Cubs were independent of the communal den.

Kinship thus influenced the association of cubs with immigrant males, regardless of offspring sex, but both kinship and sex affected sires' associations with cubs.

DISCUSSION

Spotted hyena cubs directed less intense aggression towards, and associated more closely with, their sires than control males. Immigrant males that had sired offspring associated more closely with their daughters than with control females. These behavioral biases suggest that both sires and their offspring recognized each other as kin.

There are two accepted mechanisms of kin recognition (Waldman et al. 1988): indirect kin recognition via associative cues or direct kin recognition via phenotype matching (Holmes & Sherman 1982), both mechanisms are subject to error. Errors in kin recognition that lead to errors in nepotism are analogous to errors in hypothesis testing (Reeve 1989); individuals may commit errors of acceptance and treat an unrelated animal as if it were kin, or commit errors of rejection and treat a related animal as if it were unrelated (Keller 1997). If spotted hyena sires categorized cubs nursing from former mates as their own cubs, they would often err. Copulations are rarely observed in *Crocuta* (Kruuk 1972; Szykman 2001; East et al. 2003), but observed copulations are often infertile (Szykman 2001;

East et al. 2003). In addition, multiple males have been observed mating with a single female during one estrous period (Szykman 2001; East et al. 2003), and multiple paternity occurs in at least 20% of twin litters (Engh et al. 2002; East et al. 2003). Thus, copulation provides male spotted hyenas with inaccurate cues for paternity. Therefore, even though cubs nurse for more than a year (Holekamp et al. 1999b), males would often categorize unrelated cubs as their own offspring if they used associative cues to classify cubs as progeny. Similarly, there are few obvious associative cues available for cubs to use in recognizing their sires, as there is neither overt paternal care (Mills 1990) nor any prolonged pair bond or significant degree of mate fidelity in this species (Szykman et al. 2001; East et al. 2003; Chapter Four). Therefore, both spotted hyena cubs and sires would often commit errors of acceptance if they used associative cues to recognize each other. However, mutual recognition by spotted hyena offspring and sires, as observed here, is consistent with the hypothesis that sire-offspring recognition in *Crocuta* is achieved through direct kin recognition via phenotype matching. It seems likely that this phenotype matching involves an innate, rather than a learned, template. Cubs could conceivably bias their behavior towards sires in reaction to subtle cues in the sires' behavior, but it seems that sires must refer to an innate template, as copulation does not guarantee production of offspring in this species.

The superior social rank of spotted hyena cubs over their sires creates a situation rare among mammals: filial nepotism can be stronger than paternal nepotism in spite of cubs' immaturity and inexperience. Filial nepotism among spotted hyenas may not be as obvious as in species where offspring help their parents rear younger siblings, but spotted hyenas nonetheless do favor their sires. Young hyenas directed less intense aggression towards their sires than towards unrelated immigrant males, and associated more closely with their sires than with control males; these two phenomena may be inter-related. That is, due to the fission-fusion nature of spotted hyena society, dispersive behavior may often be used to cope with aggression (Wahaj et al. 2001), resulting in weaker associations.

Offspring are equally related to their mothers and sires, yet cubs did not associate with their sires as closely as with their mothers. In addition, although cubs showed less intense aggression towards their sires, they did not otherwise bias their behavior towards their sires as they do towards their maternal relatives (e.g., Wahaj et al. 2003). Sires do not exhibit obvious paternal nepotism; their low social status may preclude nepotism of the nature and scale of maternal nepotism and nepotism between siblings (Mills 1985; East et al. 1993; Holekamp et al. 1997a; Wahaj et al. 2003). Thus, sire-cub interactions show that relatedness and recognition are not sufficient for the expression of nepotism: opportunity and the behavior-specific ratio of benefits to costs must favor the nepotist (Waldman et al. 1988; Sherman et al. 1997; Mateo 2002).

Why do spotted hyena immigrant males associate more closely with their daughters than with their sons or with unrelated individuals? One possibility is that immigrant males associate with their daughters as an investment in their own future reproductive success. This type of investment occurs in some primate species (reviewed in Whitten 1987); for example, male Barbary macaques (*Macaca sylvanus*; Ménard et al. 2001) take care of infants as an investment in future, rather than past, mating success. However, mate fidelity does not occur in spotted hyenas (Engh et al. 2002; East et al. 2003); paternal nepotism does not increase mate fidelity by female spotted hyenas. Rather than functioning in mate choice, perhaps association by sires with their daughters represents an investment in social bonds that are more likely to have long-term benefits than are social bonds with sons. If sire-daughter associations most benefit the sires, sires should initiate and maintain the associations; I do not yet know whether this is so or whether the apparent influence of cub sex on sire-cub association indices is but an artifact of small sample sizes.

Spotted hyenas recognize their sires, their offspring, and maternal and paternal siblings (Wahaj et al. 2003). Nepotism towards both maternal and paternal kin has been seen in some mammals with multi-male, multi-female matrilineal societies (e.g., Alberts 1999; Widdig et al. 2001; Smith et al. 2003) but not in others (e.g., Kuester et al. 1994; Paul et al. 1996); true paternal care is rare in these societies (but see Buchan et al. 2003). Differences among species in patterns of nepotism may be the result of differences in kin recognition abilities, variation in the relative costs and benefits of nepotistic behaviors, or variation in the availability of opportunities for nepotism. Although the fundamental asymmetry in physical maturity and experience holds true between spotted hyena sires and their offspring, the social system of *Crocuta* reverses the typical asymmetry in paternal and filial nepotism: paternal nepotism is weaker than filial nepotism.

CHAPTER FOUR

BEHAVIORAL STRUCTURING OF RELATEDNESS IN THE SPOTTED HYENA (*CROCUTA CROCUTA*) SUGGESTS DIRECT FITNESS BENEFITS OF CLAN-LEVEL COOPERATION

INTRODUCTION

Cooperation occurs when two or more individuals act together, achieving a common goal (Boesch 2003). Three broad individually-based hypotheses offer explanations for the evolution of cooperation. First, by-product mutualism occurs when each individual cooperates to increase its own direct fitness without regard for the fitness of others, but the act of cooperation also increases the fitness of the other participants (Brown 1983). Second, cooperation may also be favored through selection for reciprocity: cooperators gain net fitness benefits over repeated interactions, but alternate gains and losses of fitness in each interaction (Trivers 1971). Third, kin selection favors cooperation when an individual reaps net fitness benefits by cooperating with kin to enhance indirect fitness, despite incurring a direct fitness cost (Hamilton 1964; Wilson 1975).

Relatedness is a critical determinant of fitness gains from cooperative behavior. If there is little variation in relatedness within a group, then kin selection is unlikely to explain variation in cooperative behaviors among group members. Similarly, if relatedness is low within a group then indirect fitness, via kin selection on nepotism, is unlikely to play a strong role in the maintenance of cooperation. High relatedness within a group is not sufficient evidence of kin selection for cooperative behaviors (Queller 1992), but the presence of some closely related individuals is necessary for kin selection to occur.

A comparison of relatedness in social groups across mammalian taxa would allow qualitative assessment of the potential for indirect fitness, and kin selection, to play a role in the maintenance of cooperative behaviors. Data on genetic relatedness and behavioral interactions within and among social groups are limited, and most readily available for primates (e.g., Altmann et al. 1996; de Ruiter & Geffen 1998). Patterns of relatedness are less well known for gregarious mammalian carnivores, many of which often cooperatively capture prey, rear young, and defend resources against competitors (e.g., Geffen et al. 1996). Observational studies suggest that mean relatedness and variance in relatedness may sometimes be quite high within carnivore groups, which creates the opportunity for relatedness to influence cooperative interactions (Gompper & Wayne 1996). However, behavioral observations may be biased (Hughes 1998), so accurate assessments of relatedness are necessary in studies of cooperation (Gompper & Wayne 1996).

Spotted hyenas (*Crocuta crocuta*) are gregarious carnivores that live in large complex groups called clans (Kruuk 1972). Each clan contains one or more matrilines of adult females and their descendants, and one or more adult immigrant males, which were born elsewhere. Although solitary females searching for food may trespass into space defended by other clans (Hofer & East 1993b), females generally spend their lives within the territory of their natal clan. By contrast, males disperse from their natal clans after puberty (Frank 1986b; Hofer & East 1993c; Hofer & East 1993b; East & Hofer 2001; Boydston et al. 2003b). Clan-mates cooperate to defend the borders of their group territory from conspecifics, and also to defend food resources from conspecifics and lions (*Panthera leo*; Kruuk 1972; Mills 1990; Henschel & Skinner 1991; Hofer & East 1993c; Smale et al. 1997; Boydston et al. 2001). In East Africa, three times as many clan-mates participate on average in these risky cooperative defensive activities than in other group behaviors, including hunting (Kruuk 1972; Henschel & Skinner 1991; Hofer & East 1993c; Holekamp et al. 1997b; Boydston et al. 2001). It is known that maternal kin cooperate during group hunts (Mills 1985), but relatedness among hyenas participating in other cooperative behaviors is poorly understood (e.g., Owens & Owens 1984; Mills 1985).

Each spotted hyena clan is structured by a rigid linear dominance hierarchy (Kruuk 1972; Tilson & Hamilton 1984; Frank 1986b) that determines priority of access to food by each clan member. High-ranking hyenas have better access to food and greater reproductive success than do low-ranking individuals (Frank 1986b; Holekamp et al. 1996). Offspring attain social ranks just below those of their mothers (Holekamp & Smale 1991; Engh et al. 2000), so maternal kinship has important fitness implications. Maternal relatedness may also have more subtle fitness effects: relationships among matrilineal kin are more affiliative, cooperative, and stable than are relationships among hyenas that are not maternally related (Holekamp & Smale 1991; East et al. 1993; Smale et al. 1993; Engh et al. 2000; Wahaj et al. 2001). All assignment of clan-mates as kin or non-kin in earlier studies of *Crocuta* was based solely on maternal kinship inferred from observations (e.g., Holekamp et al. 1999a; Wahaj et al. 2001; Engh 2002; Wahaj & Holekamp 2003). However, molecular methods have recently been developed to identify paternal kin in spotted hyenas

(Engh et al. 2002; East et al. 2003). These methods also allow me to estimate relatedness values among hyenas, and to use the values to test predictions of the hypothesis that kin selection favors group-level cooperation among spotted hyenas. This hypothesis predicts, for example, that relatedness should be high among matrilines as well as within them, because members of multiple matrilines join forces in defending food and territory borders from encroaching competitors (e.g., Boydston et al. 2001).

The primary goal of this chapter was to assess whether there are opportunities for kin selection to favor cooperation among *C. crocuta.* I therefore describe and contrast patterns of relatedness within and among spotted hyena matrilines and clans, and then consider whether these patterns are consistent with the predictions of kin selection or those of other adaptive hypotheses. The secondary goal of this chapter was to compare patterns of relatedness in *C. crocuta* to those observed in other carnivores and primates living in groups containing multiple adults of each sex.

METHODS

Longitudinal analysis of relatedness within a single clan

I used intensive observations of one large clan of spotted hyenas, the Talek clan, which defends a 70 km^2

territory in the Masai Mara National Reserve of Kenya, from 1988 through 2001. Identities, sexes, and ages of all natal members of this clan were known (Frank et al. 1990; Holekamp & Smale 1998), and ages of hyenas not born in the clan could be accurately estimated (Chapter One). Natal animals of both sexes, and immigrant males remaining in the clan for at least six months, were considered residents; other immigrant males and trespassing females were considered transients (Engh et al. 2002).

Each hyena's social rank in the Talek clan was based on its position in a matrix ordered by appeasement displayed during agonistic encounters (Martin & Bateson 1988; Engh et al. 2002). Maternally related hyenas have adjacent social ranks in the clan's hierarchy (Holekamp & Smale 1991; Engh et al. 2000), so members of each Talek matriline were also grouped and ranked, as a matriline, relative to other matrilines.

I estimated relatedness (*R*; Queller & Goodnight 1989) from genotypic data. Spotted hyenas were anesthetized with Telazol (W. A. Butler Co., 6.5 mg/kg) administered in a plastic dart via a CO_2-powered rifle. Blood was collected from all Talek animals darted ($n = 201$), and *R* was calculated for four types of pairs of Talek animals: mother-cub, sire-cub, full-siblings, and half-siblings.

Members of these pairs of close kin were identified from known pedigrees (e.g., Engh et al. 2002).

To evaluate the extent to which maternal relatedness was diluted across generations by paternal gene flow, I determined *R* among the first sampled female in each matriline and all of her descendants, and among the same matriarchs and all other clan-mates, for every Talek matriline sampled across at least 3 generations ($n = 6$). Pairwise data points were not independent (Dietz 1983), so I used permutation tests as in Ratnayeke et al. (2002) to compare mean *R*-values across generations.

To examine the influence of matriline membership on relatedness, I delineated matrilines ($n = 13$) in the Talek clan from nursing observations and genetically confirmed maternity (Engh et al. 2002). I then used pairwise permutation tests to compare mean *R* among matrilines of different ranks. I used *post-hoc* Spearman's rank correlation coefficients (r_s) and Pearson's correlation coefficients (r_p) to look for effects of confounding variables on the association between matriline rank and mean *R*.

Some matrilines considered distinct might have been subunits of matrilines that fissioned prior to the initiation of this study. If so, across matrilines I would see the highest *R*-values among members of matrilines adjacent in rank, because subunits of matrilines presumably retain their relative social ranks. To seek evidence of past matriline fission events, I evaluated the relationship between rank distance and relatedness among matrilines. The matriline rank distance among members of the same matriline was set at zero, the matriline rank distance among members of matrilines adjacent in rank was set at one, etc. I assessed the correlation between *R*-values and matriline rank distances by Mantel's test (1967) with program Passage Version 1.1 (Rosenberg 2001). I used *post-hoc* pairwise permutation tests to determine whether a significant Mantel's result was due to the contrast between relatedness at a rank distance of zero and relatedness at other rank distances. I also used a permutation test to compare *R* among all Talek natal animals (83 females, 76 males) to *R* among all Talek resident immigrant males (n = 42).

Cross-sectional analysis of multiple hyena clans

To assess relatedness among clans, an intensive darting program was conducted during the summer of 2000 throughout an area of the reserve covering approximately 800 km^2 (Figure 4.1). Adult females and subadults were preferentially darted, although adult males were also sampled. Each sampled hyena was marked with a uniquely

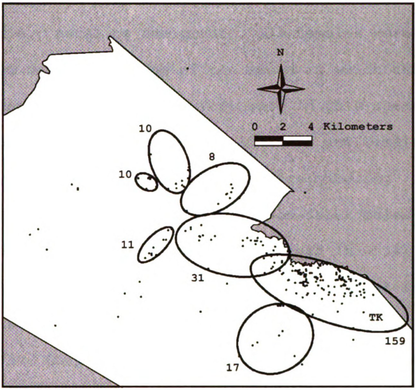

Figure 4.1—Darting locations of sampled spotted hyenas (*n* = 335) in southwest Kenya. Shaded regions lie outside of the Masai Mara National Reserve. Ovals enclose darting locations of animals sharing a communal den and therefore assigned to the same clan, for those clans with at least 8 natal animals sampled. Areas not enclosed by ovals are nonetheless defended by clans. Matrilineal relationships were known within the Talek (TK) clan from long-term observations, and confirmed with molecular analyses (Engh *et al.* 2002). Sample sizes indicate the number of natal animals sampled.

numbered ear-tag (Jumbo Rototags, Dalton I.D. Systems Inc.), and I inferred its clan membership and dispersal status (i.e., natal or immigrant). All females were considered natal residents of the areas in which they were darted; female emigration is very rare in this species (Holekamp et al. 1993; Smale et al. 1997) and females in the study region rarely trespass into neighboring territories (Boydston et al. 2001). Non-Talek males estimated to be younger than 24 months old (n = 14) were deemed pre-pubertal natal residents, as dispersal in this species is considered to occur after this age (e.g., Smale *et al*. 1997; Chapter One). Non-Talek males estimated to be older than 47 months (i.e., average age at dispersal from Talek plus one SD of my age estimation error, n = 26) were considered to be immigrants to the areas in which they were darted if they were seen there in association with one or more natal animals (n = 25). I excluded males estimated to be between 24 and 47 months of age (n = 7) because I could not infer their dispersal status. I defined clans from observations of eartagged animals visiting the same communal den. I considered no territorial borders to exist between members of the same clan, one border to exist between members of adjacent clans, and so forth. In total 176 individuals born outside the Talek clan were sampled;

76.1% of these were natal residents of clans other than Talek.

I calculated *R* for all possible pairs of animals natal to any clan (*n* = 261), and I used the spatial juxtaposition of hyena clans (Figure 4.1) to estimate the minimum number of territorial boundaries separating members of each pair. I then used Mantel's test to assess the relationship between *R*-values within pairs of hyenas and the number of clan borders separating members of each pair. *Post-hoc* pairwise permutation tests were used to determine whether significant Mantel's results were due to a contrast between *R*-values within clans and *R*-values among clans.

DNA extraction and microsatellite amplification

DNA was extracted in the field from blood with Puregene kits (Gentra Systems Inc.) and stored in liquid nitrogen (Engh et al. 2002). I amplified ten autosomal microsatellite loci (i.e., CCr01, CCr05, CCr07, CCr11, CCr12, CCr13, CCr14, CCr15, CCr16, CCr17; GenBank Accession nos. AY394080, AY394083, AY394084, AF180491-AF180497) and one X-linked microsatellite locus (i.e., CCrA3; GenBank Accession no. AY394085) using conditions described previously (Libants *et al.* 2000; Engh 2002). Tests for Hardy-Weinberg equilibrium were conducted with program Genepop Version 3.3 (Raymond & Rousset 1995), and indicated that all autosomal loci were in Hardy-Weinberg equilibrium ($p > 0.05$). Additional information on loci is available in the Molecular Ecology Primer Database at http://tomato.bio.trinity.edu/home.html.

Estimating pairwise relatedness and statistical analyses

I calculated allele frequencies with the program Cervus Version 2.0 (Marshall et al. 1998), and used program Relatedness Version 5.0 to estimate *R* (Queller & Goodnight 1989). *R* is biased when related animals contribute to the estimation of population allele frequencies (Queller & Goodnight 1989; Altmann et al. 1996). To counter potential bias, sampling included as many animals as possible that were unlikely to be related, and I calculated *R*-values using global allele frequencies estimated over the entire sample of 335 hyenas. Because nearly all hyena cubs are sired by immigrants (Engh et al. 2002), and thus nearly every clan member receives 50% of its alleles from another clan, use of global allele frequencies seemed most appropriate here.

Rather than focusing on the absolute magnitude of my estimated *R*-values, I specifically focused on the patterns among those estimates. Because *R*-values are influenced by the number of loci utilized (Altmann et al. 1996; Blouin et al. 1996; de Ruiter & Geffen 1998), I included only animals genotyped at more than eight loci. To avoid spurious relationships from small sample sizes I estimated within-group relatedness only for Talek matrilines ($n = 8$ of 13 total) and for clans ($n = 7$ of 11 total) from which I had samples from at least eight natal animals.

Results were summarized as $\bar{x}$ ± SE, and differences among groups were considered significant when $p < 0.05$. The significance of multiple comparisons using any one data set was evaluated after applying the sequential Bonferroni adjustment (Rice 1989). Sample sizes indicate numbers of pairwise R-values unless stated otherwise.

RESULTS

Longitudinal analysis of relatedness within a single clan

Patterns of relatedness within the Talek clan reflected known genealogical relationships (Figure 4.2), although mean R-values of mothers and cubs (0.446 ± 0.016, $n = 73$), sires and cubs (0.442 ± 0.019, $n = 71$), full-sibling pairs (0.439 ± 0.034, $n = 16$), and half-sibling pairs (0.239 ± 0.013, $n = 244$) were all slightly below the coefficients of relatedness expected from known pedigrees (e.g., Figure 4.3). Surprisingly, mean R-values were no higher among all pairs of natal animals (0.011 ± 0.002, $n = 12{,}561$) than

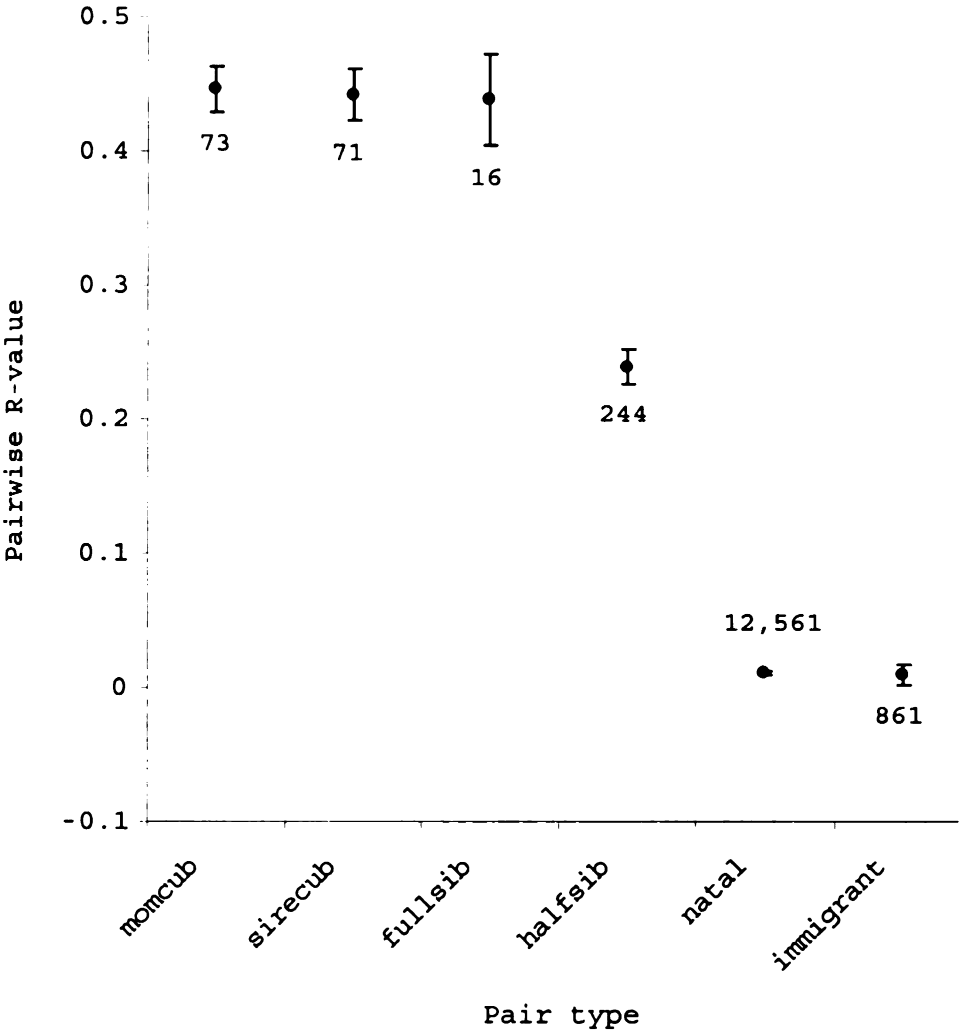

Figure 4.2—Pairwise *R*-values within six types of pairs of resident Talek hyenas: mothers and cubs (momcub), sires and cubs (sirecub), full-sibling pairs (fullsib), half-sibling pairs (halfsib), any two natal animals, and any two resident immigrants. Sample sizes indicate number of *R*-values and values are presented as $\bar{x}$ ± SE.

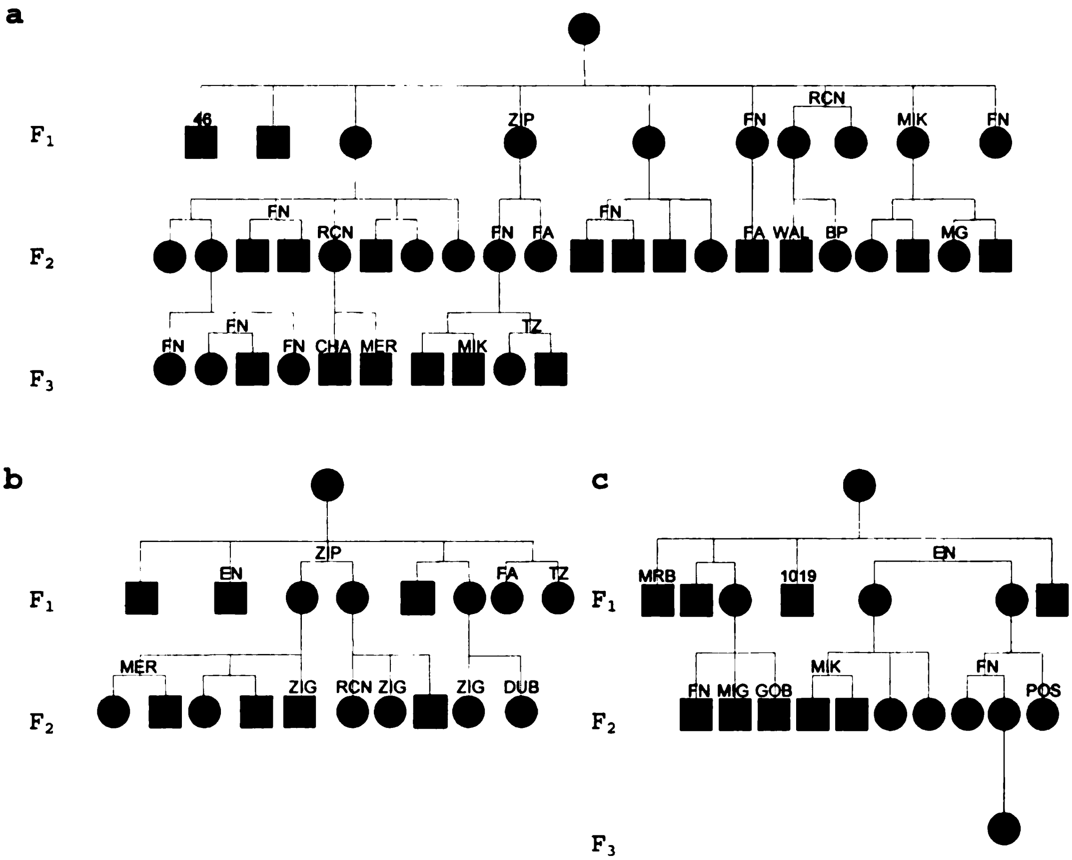

Figure 4.3—Pedigrees of sampled Talek hyenas from the (a) highest-ranked matriline, (b) second-ranked matriline, and (c) third-ranked matriline with average *R*-values of 0.076 ± 0.008, 0.256 ± 0.016, and 0.136 ± 0.017, respectively. Abbreviations indicate known sires, squares represent males, and circles represent females.

among immigrant animals (0.009 ± 0.007, $n = 861$, $p = 0.447$; Figure 4.2).

R-values declined across generations within a matriline, but remained higher between matriarchs and their descendants than between matriarchs and clan-mates from other matrilines (Figure 4.4). The mean *R*-value between the first sampled female in each matriline ($n = 6$) and their descendants dropped below values predicted from pedigrees after only one generation, yet across three generations these *R*-values (0.09 ± 0.05, $n = 11$) were still significantly higher ($p = 0.017$) than those between these matriarchs and members of other Talek matrilines (-0.05 ± 0.007, $n = 818$).

Reproductively successful males ($n = 19$) sired cubs (3.37 ± 0.754 cubs) from multiple matings (2.89 ± 0.58 matings), with females in multiple matrilines (2.0 ± 0.242 matrilines). Successful males were not constrained to mating with females from only one generation (1.21 ± 0.122 generations) within matrilines (e.g., male FN in Figure 4.3a), but this influence on relatedness within matrilines was more than countered by the effect of males mating in more than one matriline (e.g., FN in Figures 4.3b and 4.3c). That is, paternal gene flow diluted relatedness

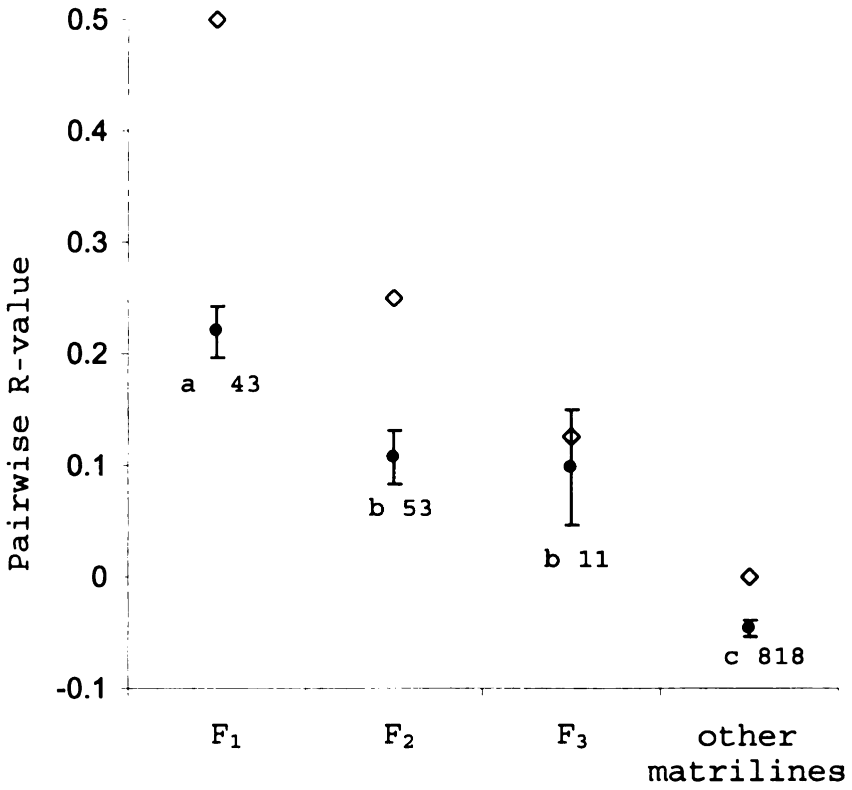

Figure 4.4—Decline in *R*-values across generations within and between Talek matrilines. Predicted (pedigree) coefficients of relatedness (open diamonds) and observed pairwise *R*-values (filled points) between the first sampled female in each Talek matriline for which at least three generations were sampled ($n = 6$), and all her descendants or clan-mates in other matrilines. Letters indicate significant differences ($p < 0.05$) based on pairwise randomization analyses, corrected for multiple comparisons. Other notation is as in Figure 4.2.

across generations within matrilines, but did not overwhelm the influence of maternal gene flow. On average, then, there should be a larger indirect fitness benefit from cooperating with distant kin from one's own matriline (e.g., great-grand-offspring) than with clan-mates from other matrilines.

Although within-matriline relatedness varied among matrilines of different rank, matriline rank did not have a consistent influence on relatedness within Talek matrilines (Figure 4.5). Mean *R* was surprisingly high within the 19 animals of the second-ranked matriline, as it was approximately equal to that expected among half-siblings. I might expect variation in relatedness within matrilines to be negatively correlated with the number of reproducing individuals, or with reproductive skew within matrilines. Although 8 of 19 animals in the second-ranked matriline were the offspring of females who happened to be full siblings (Figure 4.3b), 7 of 19 animals in the third-ranked matriline (Figure 4.3c) were also the offspring of full sisters yet the resulting mean *R*-value was significantly lower than that in the second-ranked matriline (Figure 4.5). Variation among matrilines in within-matriline relatedness was not due to any of several potential confounders: the number of females reproducing within

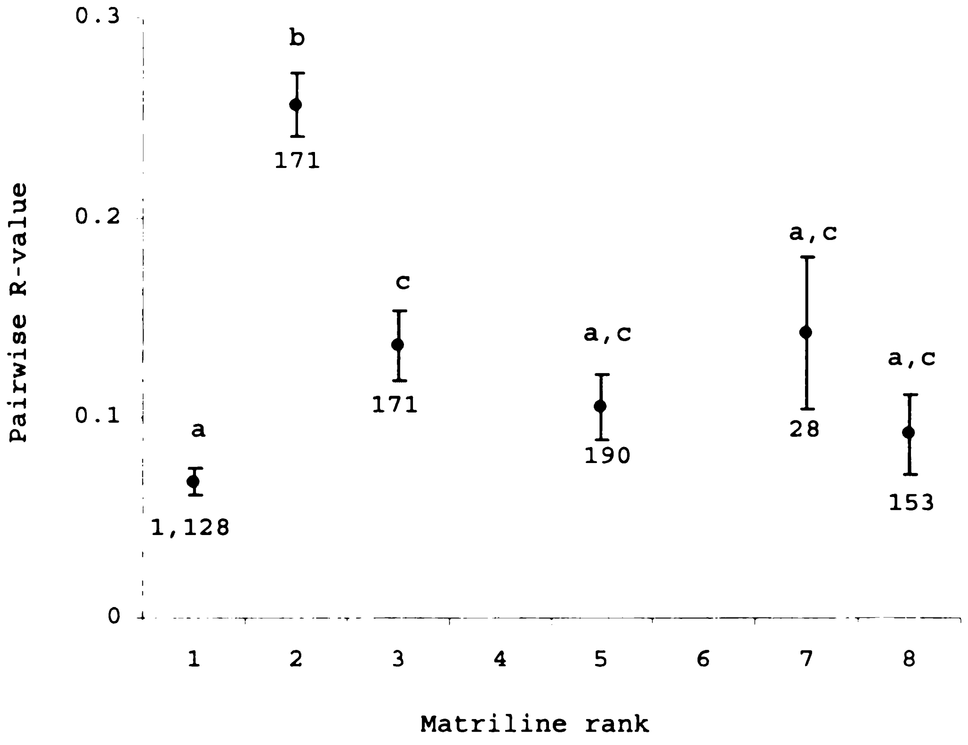

Figure 4.5—Pairwise *R*-values within matrilines of different social rank within the Talek clan. Notation is as in Figure 4.4.

matrilines (r_s = -0.441, n = 6, 0.5 > p > 0.2), the proportion of cubs produced by a single female within each matriline (r_p = -0.449, n = 6, p = 0.372), the number of males siring cubs within each matriline (r_s = -0.232, n = 6, p > 0.5), the proportion of cubs sired by a single male within each matriline (r_p = -0.036, n = 6, p = 0.946), the relatedness of males siring cubs within a matriline (r_p = 0.062, n = 6, p = 0.908), the number of generations sampled within a matriline (r_p = -0.554, n = 6, p = 0.254), or matriline-specific sample size (r_s = -0.493, n = 6, 0.5 > p > 0.2). In summary, *R* was typically lower within entire matrilines than among close kin, and variation in matriline-specific *R* was determined neither by matriline rank nor by matriline-specific reproductive skew in either sex, or to variation in sampling. The opportunities for kin selection are therefore not greater within high- than low-ranked matrilines.

Patterns of relatedness among matrilines indicated that matrilines were not homogeneous within the Talek clan (Figure 4.6). That is, pairwise *R*-values among matrilines varied with rank distance (Mantel's r = -0.1176, n = 8,551, p = 0.023), and were highest within matrilines. However, relatedness did not follow a consistent pattern across other matriline rank distances (Figure 4.6), suggesting

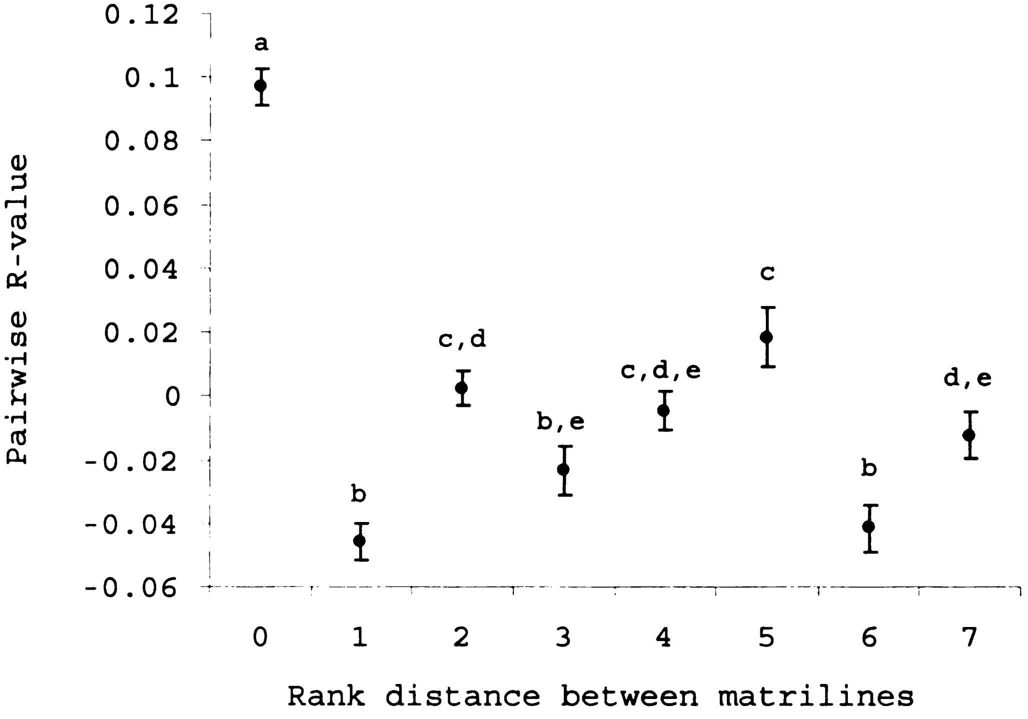

Figure 4.6—Pairwise *R*-values and matrilineal rank distances within the Talek clan; two hyenas in the same matriline have a matrilineal rank distance of 0. Sample sizes (# of *R*-values) for each matriline rank distance are as follows: n_0 = 1,756; n_1 = 1,417; n_2 = 1,452; n_3 = 740; n_4 = 1,112; n_5 = 494; n_6 = 726; and n_7 = 854. Other notation is as in Figure 4.4.

that matrilines had not fissioned in the recent past. Overall, higher relatedness within than among matrilines suggests that more indirect fitness benefits should accrue from cooperation within than among matrilines.

Cross-sectional analysis of multiple clans

R-values were negatively and significantly correlated with the number of clan borders separating natal animals (Mantel's $r = -0.074$, $n = 30{,}135$, $p = 0.001$; Figure 4.7). This association was not solely a result of the contrast of within-clan relatedness to between-clan relatedness, but indicated a gradual decline in relatedness across social barriers to dispersal. Thus, although male natal dispersal and paternal gene flow reduced within-clan relatedness, it neither masked the effects of female philopatry nor eliminated potential indirect fitness benefits of preferentially cooperating with clan-mates over members of neighboring clans.

DISCUSSION

Reproductive success, dispersal, and territoriality all influence relatedness among spotted hyenas. Bertram (1979) predicted that high dispersal rates among males and low reproductive skew among females would lead to low relatedness within clans of *Crocuta*. My results fit this prediction and suggest that relatively low reproductive

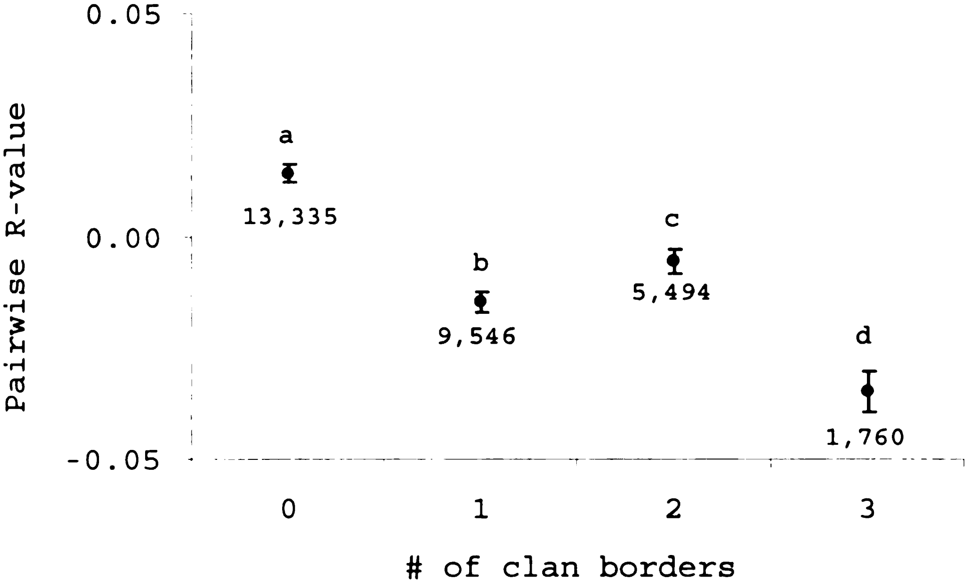

Figure 4.7—Pairwise *R*-values shown in relation to the number of clan borders separating spotted hyenas; there are no clan borders separating members of the same clan. Notation is as in Figure 4.4.

skew among male spotted hyenas further decreases relatedness within large clans.

R within social groups

The pattern of *R*-values within pairs of close kin was as expected from known pedigrees and partially as expected from male-biased dispersal (Figure 4.2), although the actual *R*-values themselves were biased slightly downwards (e.g., *R* from mother-cub pairs averaged 0.446 whereas mother-cub $r = 0.5$). I do not believe this is a result of calculating *R* based on allele frequencies that are unduly influenced by the inclusion of too many related individuals. I did not exclude known offspring from allele frequency calculations, as this would have two serious negative consequences with respect to the external validity of my work. First, I would be forced to adopt multiple different criteria for inclusion of genotypes in social units at different spatial scales, because I do not know *a priori* the kin relationships of adult females in other clans. Second, even if I did exclude known offspring from allele frequency calculations I would end up with a set of genotypes that bore little resemblance to the sampled social or genetic units. Therefore, estimates of allele frequencies made across all clans appear to be the most appropriate and unbiased reference set for my purpose here.

In any case the bias in my estimates of *R* occurs across all social levels, and should therefore not influence how I interpret variation in *R*-values among social levels.

As expected, *R*-values were lower among adult males than among adult females (Figure 4.2). Thus, I could have inferred the sex-bias in dispersal from *R*-values alone, as has been done in other species (e.g., Utami et al. 2002). The level of gene flow into social groups determines whether philopatric individuals will be more closely related than dispersed individuals. For example, de Ruiter and Geffen (1998) found that pairs of philopatric long-tailed macaques (*Macaca fascicularis*) were more highly related than immigrant pairs, even though kin sometimes dispersed together. Reduced relatedness among dispersers, relative to non-dispersers, has also been observed in Japanese macaques (*M. fuscata*; Aoki & Nozawa 1984), savannah baboons (*Papio cynocephalus*; Altmann et al. 1996), and chimpanzees (*Pan troglodytes*; Morin et al. 1994). I observed unexpectedly similar *R*-values between known natal and immigrant hyenas (Figure 4.2). This is likely due to high gene flow and relatively low reproductive skew among *C. crocuta*, as has also been reported in studies of chimpanzees (Gagneux et al. 1999; Vigilant et al. 2001) and bonobos (*Pan paniscus*; Gagneux et al. 1999; Gerloff et al. 1999; Vigilant et al. 2001).

R within and among matrilines

Relatedness within the Talek clan is structured along matrilineal lines. However, as Gompper and Wayne (1996) predicted for *C. crocuta*, the influx of paternal genes diluted relatedness across generations within Talek matrilines (Figure 4.4). Relatedness was greater within than among matrilines of spotted hyenas (Figure 4.6), as in savannah baboons (Altmann et al. 1996), long-tailed macaques (de Ruiter & Geffen 1998), and gray mouse lemurs (*Microcebus murinus*; Wimmer et al. 2002). High-ranking macaque matrilines were also more closely related to each other than were lower ranking matrilines in the same group, apparently due to rapid growth and fissioning of high-ranking matrilines within a group (de Ruiter & Geffen 1998). This was not the case for matrilines within the Talek clan. Although higher-ranking female hyenas do have greater reproductive success than low-ranking females (Holekamp et al. 1996; Holekamp & Smale 1999), matriline fission is apparently less common among hyenas than macaques (Holekamp et al. 1993).

There was no consistent decline in within-matriline relatedness as matriline rank declined in the Talek clan

(Figure 4.5). This differs from the pattern observed in long-tailed macaques, where relatedness was greater within higher- than lower-ranking matrilines due to male reproductive skew and mate choice for high-ranked females, who were of greater reproductive value than low-ranked females (de Ruiter & Geffen 1998). Rank also has strong effects on female reproductive success among *C. crocuta* (Holekamp et al. 1996; Holekamp & Smale 1999), so it seems doubtful that interspecific differences in rank-related female reproductive skew explain the interspecific differences in relatedness patterns. However, these differences may arise from greater control of reproduction by female spotted hyenas than by female long-tailed macaques, due to the dominant social status of females relative to breeding males among spotted hyenas (Engh et al. 2002).

R and dispersal across territorial boundaries

The decline in average *R* across hyena clan boundaries (Figure 4.7) is similar to declines in *R* across territorial boundaries of chimpanzees (Vigilant et al. 2001) and African lions (Spong & Creel 2001; Spong et al. 2002), and suggests that most successful dispersal by male *C. crocuta* occurs to nearby clans. Such dispersal would match the general theoretical prediction of Waser (1987) and behavioral observations on *C. crocuta* made by Smale *et al.* (1997) and Boydston (2001). If males disperse to nearby clans, they may encounter previously dispersed male relatives there. I do not yet know if the relatedness of potential immigrants to resident immigrants affects the probability of settling in a particular clan, but dispersing male hyenas are more likely to be attacked by resident males than females (Boydston et al. 2001), and kinship has been found to influence immigration in other social mammals (e.g., *Lycaon pictus*; Girman et al. 1997; vervet monkeys, *Chlorocebus aethiops*; Cheney & Seyfarth 1983).

Relatedness and cooperation among spotted hyenas

Genetic relatedness should influence social behavior because animals able to interact differentially with kin and non-kin would have higher inclusive fitness than animals unable to do so (Hamilton 1964). Relatedness was higher within than among Talek matrilines (Figure 4.6), so it would be more genetically profitable to cooperate with even distant matrilineal kin than with members of other matrilines in the clan. Indeed, Mills (1985) found that hyenas were more likely to cooperate with members of the same matriline than other matrilines while hunting. However, members of multiple hyena matrilines frequently cooperate in defensive coalitions against lions and against hyenas in other clans (Kruuk 1972; Mills 1990; Henschel & Skinner 1991; Hofer & East 1993c; Smale et al. 1997; Boydston et al. 2001). If such risky cooperative behavior is to be explained by kin selection, then individuals engaging in these activities should be closely related. I found that pairs of hyenas born in the same clan are, on average, unrelated. Because clan-mates were almost certainly sired by immigrant males (Engh et al. 2002), these unrelated clan-mates may often cooperate against their own paternal aunts and cousins from other clans. Group-level cooperation with nonkin against kin seems unlikely to be the result of cooperation driven by kin selection, yet it occurs in spotted hyenas and in gray wolves (Lehman et al. 1992). I did not measure the fitness benefits and costs of clan-level cooperation, but the patterns of relatedness among spotted hyenas suggest that there is little opportunity for kin selection to maintain clan-level cooperation. Although kin selection has been supported as an explanation for the evolution of cooperative behaviors among some carnivores (Packer et al. 1991; Gompper & Wayne 1996), cooperation without kinship has been seen in other social carnivores (Russell 1983;

Clutton-Brock & Vincent 1991; Lehman et al. 1992; Grinnell et al. 1995; Forbes & Boyd 1996), as in *Crocuta*.

It has been suggested that natural selection has favored grouping by female *C. crocuta* to defend territories containing a stable supply of prey (Kruuk 1972; Henschel & Skinner 1991; Boydston et al. 2001). If true, this clan-level cooperation would be an example of by-product mutualism (Brown 1983). Indeed, Henschel and Skinner (1987) suggested as much even for small clans composed of highly related individuals. Reciprocity (Trivers 1971) might also explain clan-level cooperation in the spotted hyena, although reciprocity appears to not play a major role in group-level cooperation in another large carnivore, the African lion (Grinnell 2002). Chesser *et al.* (1993) argued that, if the direct fitness advantages of group living are high, then there is selection pressure for individuals to join groups, regardless of their relatedness to other group members. This leads to low coancestry within groups; I conclude from the low relatedness within hyena clans that there may be strong direct fitness advantages to group living in *C. crocuta*, either through by-product mutualism or through reciprocity.

APPENDIX A

The following measurements were taken from immobilized or necropsied *Crocuta*. Measurements followed by numbers in parentheses were retained for entry into multiple linear regression analyses, as the intermediate stage of model construction, with the number(s) indicating the subsequent final model equation(s) provided in the text. All dental measurements were taken on the buccal tooth surface with calipers to within 0.1 mm. Cranial and postcranial measurements were measured with cloth tape measures to within 0.1 cm. Total mass was taken on a digital scale to within 0.1 kg. Broken or decayed teeth were not measured, and crown heights were not recorded if gum erosion precluded accurate measurement. Unless otherwise noted, dental and limb measurements were taken on only 1 side of the body.

Dental measurements

HtLtp3 and HtRtp3: Minimum crown height of the left and right lower 3rd premolars, respectively, measured from the occlusal surface to the base of the enamel as in Kruuk's (1972) Figure 7.

p3: The mean of HtLtp3 and HtRtp3 (2-4).

OCCLtp3 and OCCRtp3: Maximum anterior-posterior length of the occlusal surface of the left and right lower 3rd premolars. OCCp3: The mean of OCCLtp3 and OCCRtp3 (2-4).

C1: Minimum crown height of the upper canine, measured from the apex to the base of the enamel (1-3).

c1: Minimum crown height of the lower canine, measured from the apex to the base of the enamel (1-4).

INC: Maximum width of the medial four upper incisors (1-3).

P2: Maximum anterior-posterior length of the upper 2nd premolar, measured at the base of the enamel (2-3).

P3: Maximum anterior-posterior length of the upper 3rd premolar, measured at the base of the enamel.

P4: Maximum anterior-posterior length of the upper 4th premolar, measured at the base of the enamel.

p2: Maximum anterior-posterior length of the lower 2nd premolar, measured at the base of the enamel.

p3: Maximum anterior-posterior length of the lower 3rd premolar, measured at the base of the enamel.

p4: Maximum anterior-posterior length of the lower 4th premolar, measured at the base of the enamel.

UPROW: Maximum anterior-posterior length of the upper tooth row, measured from the most anterior edge of the upper canine to the posterior edge of the upper 4th premolar (2-3).

LOROW: Maximum anterior-posterior length of the lower tooth row, measured from the anterior edge of the lower canine to the posterior edge of the 3rd lower premolar.

Cranial measurements

HEAD: Maximum distance from the tip of the rhinarium to the posterior edge of the external occipital protuberance, measured along the sagittal midline (1).

TOPCREST: Perpendicular distance from the sagittal crest to the widest point of the zygomatic arch (1).

BACKCREST: Maximum distance from the posterior edge of the external occipital protuberance to the widest point of the zygomatic arch.

EAR: Maximum distance from the notch at the posterior edge of the tragus to the apex of the pinna (1).

Post-cranial measurements

BODY: Distance from the posterior edge of the external occipital protuberance to the base of the tail, measured along the spine.

TAIL: Distance from the base of the tail to the posterior tip of the most caudal vertebra, measured along the dorsal midline. HEADCIRC: Maximum circumference of the head, measured at the widest point of the zygomatic arches, perpendicular to the sagittal crest.

NECKCIRC: Circumference of the neck, measured midway between the shoulders and the head (1).

SHOULDER: Maximum distance from the bottom of the plantar pad of the forepaw to the cranial angle of the scapula, measured with the foreleg extended perpendicular to the spine.

SCAPULA: Maximum distance from the cranial angle of the scapula to the anterior tip of the acromion (1).

UPLEG: Maximum distance from the anterior tip of the greater tuberosity to the posterior tip of the lateral epicondyle of the humerus.

LOWLEG: Maximum distance from the tip of the olecranon of the ulna to the tip of the accessory carpal.

FRONTFOOT: Maximum distance from the posterior tip of the accessory carpal to the anterior edge of the 3rd digital pad, measured along the palmar midline (1).

HINDFOOT: Maximum distance from the posterior tip of the tuber calcanei to the anterior edge of the 3rd digital pad, measured along the palmar midline.

GIRTH: Minimum circumference of the torso, measured immediately posterior of the forelegs, with the forelegs perpendicular to the body.

MASS: Total mass of the individual.

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