COMPETITION AND COOPERATION AMONG MALES IN A SEX-ROLE REVERSED MAMMAL, THE SPOTTED HYENA (*CROCUTA CROCUTA*)

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ABSTRACT

COMPETITION AND COOPERATION AMONG MALES IN A SEX-ROLE REVERSED MAMMAL, THE SPOTTED HYENA (*CROCUTA CROCUTA*)

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Sexual selection theory predicts that when females have the limiting reproductive rate, males should compete for access to those females via one of several mechanisms, such as male-male combat, sperm competition, or endurance rivalry. In this dissertation, I investigated the nature of intrasexual male competition in a sex role-reversed species, the spotted hyena (*Crocuta crocuta*), by examining each of those mechanisms. First, I tested hypotheses regarding the function of male-male combat. In most mammals, male-male combat functions to provide immediate access to females, but in spotted hyenas, females have complete control over copulation, so the benefits of intrasexual male aggression are less obvious. The results suggested that although males cannot control copulation, they use aggression to influence clan membership by restricting male immigration, which in turn affects their likelihood of siring cubs. The data likewise suggested that male aggression functions to provide access to food during competition with other males. Second, I took steps toward elucidating the importance of sperm competition in this species by describing ejaculate quality in wild male spotted hyenas and demonstrating temporal repeatability within individuals. Additionally, I found that immigrant males had significantly higher quality ejaculates than adult natal males, suggesting that adult natal males might experience reproductive suppression prior to dispersing. Third, I explored the notion that male spotted hyenas compete via an endurance rivalry by examining the relationship between tenure in the clan and annual reproductive success, and then investigated additional factors that might influence the outcome of this contest. The results indicated that immigrant males do indeed compete via an endurance rivalry, and for an immigrant to compete most effectively, he must remain in the clan and associate closely with females. However, pieces remain missing from the puzzle of male reproductive success in this species, because I found a striking quadratic effect of tenure on annual reproductive success, and the eventual decline in reproductive success remains unexplained. Finally, in addition to investigating male competition, I asked what evolutionary forces promote cooperative behavior among males. Kin selection and reciprocal altruism appeared to play no role in male-male cooperation; rather, males appeared to cooperate with one another to receive a variety of direct benefits. Overall, these results help illuminate the selective forces shaping competition and cooperation among males in a role-reversed species, and suggest how these forces might influence a male's reproductive output.

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One component of my second chapter relied on data I collected at the captive hyena colony in Berkeley, CA, which is run by Steve Glickman and is managed by Mary Weldele. Steve and Mary allowed to visit the colony on two separate occasions to collect ejaculate samples to supplement those I had collected from wild hyenas in Kenya. I appreciate their flexibility and help with that project, and I thank the other employees that helped me while I was there, especially Michelle.

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GENERAL INTRODUCTION

Intrasexual competition has been invoked as an integral component of sexual selection since the inception of sexual selection as part of of evolutionary theory (Darwin 1871). When one sex has a limiting reproductive rate, it becomes the more “choosy” sex, and the other sex must compete to be chosen (Darwin 1871; Andersson 1994; Andersson & Iwasa 1996). Because females have the limiting reproductive rate in most species, intrasexual competition is commonly referred to as “male-male competition.” Males must compete for access to females during every stage of reproduction (Andersson 1994): access to proximity to females (Bercovitch 1997), access to copulations, and access to fertilization (Parker 1970). In many mammals, intrasexual competition manifests as male-male physical combat, and is thus often easily quantified by measuring the size of weapons and the rates or intensities of aggression (Berglund et al. 1996; Qvarnström & Forsgren 1998). However, further research has demonstrated that male-male competition can also take alternative forms, including sperm competition and endurance rivalry (Andersson 1994).

Spotted hyenas as a model for testing predictions of sexual selection theory

The predictions of sexual selection theory have generally arisen from studies on species with classic sex roles (Andersson 1994), but how do these predictions change when traditional mammalian sex roles are reversed, and females are socially dominant to males? How does living at the bottom of the social hierarchy change the selection pressures affecting male reproductive success? The spotted hyena (*Crocuta crocuta*) presents itself as a desirable model for addressing these questions because it appears to represent an exception to normal mammalian “rules.” Spotted hyenas align with traditional sex roles in that females are the limiting sex and provide complete parental care (Kruuk 1972; Holekamp et al. 1996). However, hyenas are sex role-reversed in that female spotted hyenas are larger than, and socially dominant to, males (Kruuk 1972; Holekamp et al. 1996). The goal of my dissertation was therefore to test the bounds of sexual selection theory by examining a species that violates many of the classic mammalian patterns.

To date, little is known about the relationships among adult male spotted hyenas: given the position of males as subordinates in hyena society, are there cooperative dimensions to these relationships, or are they strictly competitive? The limiting reproductive rate of female spotted hyenas predicts that males should compete intrasexually for access to the females (Kruuk 1972; Andersson 1994; Holekamp et al. 1996), but the mechanisms by which males compete remain unknown. Therefore, in each of the first three chapters of my dissertation, I explored a different mechanism of intrasexual competition among male spotted hyenas. The final chapter considered the cooperative dimensions of male-male relationships in this species by investigating aggressive coalitions among males. My ultimate objective was to create a composite picture of the selective forces acting on male spotted hyenas and influencing their reproductive output.

Chapter 1: The functions of male-male aggression in a role-reversed mammal

Males in a wide range of species use agonistic displays and physical combat to contest access to females, or to contest access to the resources required to attract females (Clutton-Brock et al. 1982; Andersson 1994). Both posturing and actual combat select for sexual dimorphism across multiple traits, which typically results in male-biased body size, aggression, and weaponry (Berglund et al. 1996; Hack 1997; Qvarnström & Forsgren 1998). The function of male-male aggression in a role-reversed species lacking these classic dimorphisms is therefore less clear.

Male aggression has been largely dismissed as a selective force in spotted hyenas (East & Hofer 2001; Engh et al. 2002; but see Van Meter 2010), but has not been formally tested as a mechanism of sexual selection in this species.

In my first chapter, I compared intrasexual aggression rates among adult males to those among adult females, and demonstrated that the rates are comparable between the sexes after controlling for opportunities to act aggressively, based on the social constraints of the dominance hierarchy. I then tested three hypotheses regarding the functions of male-male aggression in this species. First, I proposed that male-male aggression functions to prevent other males from accessing females. The data did not support this hypothesis, however, as males exhibited less intrasexual aggression in the presence of adult females than when no female was present. Next, I tested the hypothesis that the function of male aggression is to restrict clan membership by driving out potential immigrants. New immigrants received significantly more frequent aggression from other immigrants than did established immigrant residents of the clan, a pattern that is consistent with this hypothesis. Finally, I asked whether male-male aggression functions to provide access to food, and found that males exhibit more frequent and more intense aggression in the presence of food than when no food is present, thereby supporting the hypothesis.

Chapter 2: Ejaculate quality in spotted hyenas: intraspecific variation in relation to life history traits

In recent decades, it has become apparent that male-male competition extends beyond contests over access to copulations, and includes competition between the sperm of rival males to fertilize a female’s ova (Parker 1970; Birkhead & Pizzari 2002; Birkhead et al. 2008). Sperm competition, which is particularly common in mammals (Gomendio et al. 1998; Dixson & Anderson 2004; Stockley 2004), can implicate numerous ejaculate traits, such as ejaculate volume, sperm concentration, number of sperm, sperm motility, and sperm length (Birkhead et al. 2008; Parker & Pizzari 2010; Montoto et al. 2011; Tourmente et al. 2011). Little is known, however, about the role of ejaculate quality in natural mammal populations, particularly in carnivores.

In Chapter 2, I documented variation in ejaculate quality among wild male spotted hyenas, and then tested hypotheses addressing the relationship between ejaculate quality and various life history traits. First, I tested the hypothesis that reproductive senescence manifests as ejaculate quality declining with age, but found no evidence for this. Then, I asked if dispersal status predicts ejaculate quality, and found that adult immigrant males have ejaculates of significantly higher quality than those of their adult natal male counterparts. This result suggests that adult natal males might experience reproductive suppression prior to dispersing from their natal clan, a hypothesis that is consistent with previous research on the effects of dispersal status in this species (Holekamp & Sisk 2003). Finally, I utilized repeated samples collected from six captive male spotted hyenas to demonstrate that individual ejaculate quality shows temporal stability.

Chapter 3: The relationship between tenure and reproductive success among male spotted hyenas

Endurance rivalry refers to competition among males to remain reproductively active for as long as possible (Andersson 1994; Judge & Brooks 2001; Lidgard et al. 2005; Higham et al. 2011). Typically, the term "endurance rivalry" is applied to species with brief, seasonal breeding periods, which makes spotted hyenas appear ineligible, as they breed year-round (Kruuk 1972; Holekamp et al. 1996). However, previous research has indicated that an immigrant male's reproductive success may be affected by his tenure in the clan (East & Hofer 2001; Engh et al. 2002), a trend that hints at a less conventional, but equally viable, form of endurance rivalry. The goal of my third chapter was to investigate the nuances of this potential endurance rivalry in spotted hyenas.

First, I examined how long immigrants remained in the clan before siring their first cubs, and found that, although most males did not sire cubs until they had been in the clan for over two years, if a male sired at least one cub, he typically did so in the first four years of his tenure. Then, I asked how long immigrants remained in the clan before disappearing, and found that among males that sired at least one cub, most males remained in the clan for at least four years. In contrast, males that never sired any cubs ordinarily disappeared by their fourth year in the clan. Taken together, these results suggest that immigrants might make decisions regarding whether or not to engage in secondary dispersal based on their initial reproductive success in the clan.

I then probed more deeply into various predictors of annual reproductive success among males, focusing particularly on tenure, with the goal of asking whether males compete via an endurance rivalry, and, if so, what it means to "endure," particularly given the mismatches between spotted hyenas and species engaging in more traditional endurance rivalries (Andersson 1994; Judge & Brooks 2001; Lidgard et al. 2005; Higham et al. 2011). I found that annual reproductive success among males increased with tenure for the first six years of tenure, but subsequently declined. Furthermore, I found that males that associated socially with more adult females had significantly higher annual reproductive success. However, I found no effect of how often a male actively engaged in aggressive interactions with females. Although aspects of male reproductive success in this species remain nebulous, my results appeared to be consistent with the hypothesis that male spotted hyenas compete via an endurance rivalry.

Chapter 4: Direct benefits promote aggressive coalitions among male spotted hyenas

After exploring three mechanisms of male-male competition in spotted hyenas, I then examined male-male cooperation. Cooperative behavior has perplexed evolutionary biologists since Darwin (1859) recognized that apparent altruism was in direct contradiction to his theory of evolution by natural selection, and more than 150 years later, it remains a vexing concept (Nowak et al. 2010; Abbot et al. 2011). Darwin's (1859) theory of natural selection predicts that an individual should always behave in a way that maximizes its own fitness. In contrast, a cooperative individual might act in a way that benefits another individual while incurring a cost to itself. Three primary hypotheses have been proposed to explain cooperation in the context of Darwin's theory of evolution by natural selection (West et al. 2007): kin selection, in which an individual cooperates with individuals with shared genes to obtain inclusive fitness benefits (Hamilton 1964; Maynard Smith 1964); reciprocal altruism, in which an individual cooperates with non-kin with the expectation that a commodity or service will be returned in the future (Trivers 1971; Axelrod & Hamilton 1981); and direct benefits, in which an individual cooperates in order to immediately and directly benefit from a collective outcome (Brown 1983; Maynard Smith 1983; Connor 1995).

Aggressive coalitions have commonly been used to test these hypotheses, particularly in mammals, but thus far the majority of this research has been on primates (Harcourt 1992; Widdig et al. 2000; Silk et al. 2004; but see Smith et al. 2010). Therefore, in my fourth chapter, I tested each of these hypotheses as a potential evolutionary force promoting aggressive coalitions among males in a non-primate mammal, the spotted hyena. I found no evidence for either kin selection or reciprocal altruism operating among males in this species. However, I found multiple lines of evidence supporting the direct benefits hypothesis. Males utilized coalitions to maintain the status quo in the social hierarchy, to increase their access to food, and to appeal to female preferences, although they did not seem to use coalitions to restrict other males from immigrating into the territory. These results suggest that the pursuit of direct benefits is the leading force favoring male-male coalitions in this species.

Presentation of chapters as manuscripts

The research I presented in my dissertation was the product of collaborative efforts with colleagues, and each chapter was written as an independent manuscript for publication, so hereafter, I use the term “we,” rather than “I.” Chapter 2 is currently in press at the *Journal of Mammalogy*, and I plan to submit Chapter 1 to *Animal Behaviour*, Chapter 3 to *Ethology*, and Chapter 4 to either *Animal Behaviour* or *Behavioral Ecology*.

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CHAPTER 1
THE FUNCTIONS OF MALE-MALE AGGRESSION IN A ROLE-REVERSED MAMMAL

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Abstract

The primary function of male-male aggression is widely held to be providing access to females, but its function in role-reversed species is less clear. Male-male aggression has been presumed absent in spotted hyenas (*Crocuta crocuta*), a role-reversed mammal with highly aggressive females, but has never been formally explored as a mechanism of sexual selection. Here, we first demonstrated that males display intrasexual aggression at rates similar to rates of intrasexual aggression among adult females, then tested three hypotheses suggesting functions of this aggression among males. Males aggressed significantly less in the presence of an adult female than when adult females were absent, suggesting that they do not use aggression to prevent other males from remaining in close proximity to females. New immigrants received significantly more aggression from other immigrants than did established immigrants, but new and established immigrants were treated equally by adult natal males. These results supported a hypothesis suggesting that male-male aggression functions to restrict clan membership, although the data indicated that it is immigrants, not adult natal males, engaging in aggression for this reason. Finally, a hypothesis suggesting that this behavior functions to provide access to food was supported by data showing that male-male aggression occurred significantly more frequently, and at higher intensities, in the presence than absence of food. In contrast to male-male contests in most other mammals, those in spotted hyenas appear to increase a male's access to females only indirectly, which is presumably due to the sex role-reversed nature of societal structure in spotted hyenas.

Keywords: *Crocuta crocuta*, male-male aggression, role-reversed species

Introduction

Aggression is well-documented as a behavior that can impose substantial costs to the aggressor as well as the recipient (Clutton-Brock et al. 1979; Blanchard et al. 1988; Drews 1996; Aureli 1997; Castles & Whiten 1998; Kelly & Godin 2001; Briffa & Elwood 2004; Schino et al. 2007; MacCormick et al. 2012). Despite these costs, intraspecific aggression, particularly among males, is widespread across animal taxa. Male-male aggression generally occurs during competition over access to resources, and, in most cases, the resources in question are mates (Darwin 1871; Le Boeuf 1974; Andersson 1994). Agonistic interactions often determine male dominance status (Packer 1979b; Van Noordwijk & van Schaik 1988; Haley et al. 1994; Alberts et al. 2003; Pelletier & Festa-Bianchet 2006), which has significant fitness implications, because higher-ranking males often have the best access to females and potentially also the greatest reproductive success (Le Boeuf 1974; Packer 1979b; Cowlishaw & Dunbar 1991; Haley et al. 1994; Andres et al. 2001; Alberts et al. 2003; Natoli et al. 2007). However, male-male aggression can occur over resources only indirectly related to mating access (West-Eberhard 1979; West-Eberhard 1983; Richard 1992), including food (Janson 1985; Richard 1992; Baker et al. 1993), group membership (Richard 1992), territory (Stamps & Krishnan 1997; Emlen & Wrege 2004), and nesting materials (Takahashi et al. 2001).

Little is known about male-male aggression in role-reversed species, in which females are either codominant with or dominant to males. Males of these species often lack the sexual dimorphism in body size and weaponry typically selected for by intense male-male combat

(Plavcan & van Schaik 1992; Andersson 1994; Haley et al. 1994; Mitani et al. 1996; Plavcan 1999; Surbeck et al. 2012), but this does not necessarily suggest that males of these species fail to engage in intrasexual aggression (Berglund 2005; Lawler et al. 2005). For example, various lemur species show little sexual dimorphism but do display male-male combat (Kappeler 1990; Richard 1992; Kappeler 1996; Andres et al. 2001; Lawler et al. 2005), and males in the sex-role reversed pipefish (*Syngnathus typhle*) have been shown to compete physically over access to females (Berglund 2005). However, male-male aggression in role-reversed species may be of lower intensity than in species with traditional sex roles (e.g., Richard 1992; Kelly & Godin 2001).

The functions of male-male aggression may be more nebulous in role-reversed species than in other species, in part because female dominance can preclude males from converting physical victories against other males into immediate copulations with females (Richard 1992), as sometimes occurs in species with traditional sex roles (Smuts & Smuts 1993; Clutton-Brock & Parker 1995). Furthermore, most earlier work on male-male aggression in role-reversed species has been done with primates. Our goal here is therefore to explore the functions of this behavior in a non-primate, role-reversed species, the spotted hyena (*Crocuta crocuta*).

In contrast to most mammals, spotted hyenas exhibit moderate female-biased sexual dimorphism in body size (Swanson et al. 2011), no dimorphism in weaponry (Van Horn et al. 2003), and female social dominance (Kruuk 1972). However, despite these role-reversals, sexual selection theory predicts that male hyenas should compete via some mechanism, because females have the limiting reproductive rate (Darwin 1871; Kruuk 1972; Andersson 1994; Holekamp et al. 1996; Mitani et al. 1996). Although male hyenas do not commonly engage in the life-threatening physical conflicts typical of males in other species (Andersson 1994), subtler forms of male-male aggression do exist in this species (Van Meter 2010). The possible functions of this behavior have never been explored. Here, we quantify rates of intrasexual aggression among male spotted hyenas, and then test three non-mutually exclusive hypotheses regarding the function of this behavior.

Although spotted hyenas are similar to many primate species (Alberts et al. 2003) in that males have linear dominance hierarchies that at least partially predict mating success (Engh et al. 2002), hyenas differ markedly in that these hierarchies are not determined by physical contests, but are instead dictated exclusively by tenure in the clan (Smale et al. 1997; East & Hofer 2001). Male-male aggression therefore does not function in this species to improve a male's social rank. It also cannot guarantee males access to copulations, because the unique morphology of the female reproductive tract gives females complete control over copulation (Cunha et al. 2003). Given these social and morphological limitations, male-male aggression has previously been presumed ineffective in this species (East & Hofer 2001; Engh et al. 2002). The potential functions we present here, however, could all increase an aggressor's fitness without directly influencing his social rank or immediate access to copulations.

Our first hypothesis proposes that male-male aggression functions to provide the aggressor with enhanced access to females. Although the extreme degree of female control over copulation prevents males from using agonistic contests to directly secure copulations (East & Hofer 2001; Cunha et al. 2003), perhaps males are competing for access to females less directly (Parga 2006). For example, males may use aggression to prevent other males from forming amicable relationships with females, which can be an effective mating strategy in species lacking forced copulations, such as bonobos (*Pan paniscus*) (East et al. 2003; Surbeck et al. 2012). Although female hyenas rarely choose adult males as social partners (Szykman et al. 2001), males nevertheless attempt to associate with females (East & Hofer 2001; Szykman et al. 2001), and thus may attempt to use aggression to prevent other males from doing the same. Furthermore, social contexts can influence male-male aggression: for example, Procter et al. (2012) found that selection on traits relevant to male-male combat in cactus bugs (*Narnia femorata*) was stronger when a female was present than when males were alone. In some cases, females may even incite male-male aggression in order to evaluate male quality (Cox & Le Boeuf 1977; Pizzari 2001). Previous work has also demonstrated that the presence of a sexually receptive female increases male-male competition (Cavigelli & Pereira 2000; Parga 2006; Gould & Ziegler 2007), which can result in higher rates of male-male aggression. For example, Surbeck et al. (2012) found that male bonobos, which are codominant to females, displayed significantly more aggression in the presence of a fertile female. Thus, the hypothesis that male-male aggression functions to prevent other males from accessing females predicts that male spotted hyenas should aggress more often (or at higher intensities) against other males when at least one adult female is present than when females are absent, and that this effect should be strongest when there is an estrus female present.

An alternative, even more indirect way for male hyenas to influence their access to females would be to influence clan membership. Aggressive contests among males over territory or group membership is well-documented in other species (French & Snowdon 1981; Wingfield & Marler 1988; Richard 1992), and this aggression may occur in place of competing over direct access to females (Takahashi et al. 2001), particularly in role-reversed species (Shibata & Kohda 2006). In some cases, aggression against intruders can be even more intense than intragroup aggression (French & Snowdon 1981; Ross et al. 2004; but see French & Inglett 1989). In the case of spotted hyenas, which exhibit male dispersal and female philopatry (Smale et al. 1997;

Van Horn et al. 2003), females usually choose resident males to sire their cubs (Engh et al. 2002), rather than "alien" males that are not current members of the clan. Thus, it follows that the strength of male-male competition increases as the number of resident males in a clan increases, and the composition of adult males in a clan can be viewed as an oligopolistic market in which all resident males have a mutual interest in limiting new entrants. Resident males might therefore use aggression to restrict potential immigrants from joining the clan. Intruder males attempt to immigrate into a clan with varying degrees of success (Smale et al. 1997), but their fate is typically decided within three months of their initial foray into the territory; by that time, they have either become established members of the clan, or they have failed in their attempt (Smale et al. 1997). The hypothesis that male-male aggression functions to restrict clan membership therefore predicts that immigrants in the first three months of their tenure should receive significantly more frequent or more intense aggression than more established immigrants, whose tenure exceeds three months.

Finally, male-male aggression might function to provide males with access to food. Feeding competition among spotted hyenas is quite intense (Kruuk 1972; Frank 1986b), and because access to a carcass is determined by social rank (Frank 1986b), the low-ranking positions of males in the clan's social hierarchy make food access especially difficult for them. Furthermore, because many carcasses are too large for one individual to monopolize (Watts et al. 2009), conflicts often arise at kill sites. If male-male aggression functions to improve access to food, we should expect males to aggress more often or at higher intensities in the presence of food than when no food is present.

Methods

Subject population

Our study population consisted of a wild spotted hyena clan that was continually monitored in the Masai Mara National Reserve, Kenya, from 1988-2009. Clans typically contain 50-90 individuals, all of whom can be identified by their unique spots and other markings (Frank 1986a). We determined the sex of each individual using the dimorphic morphology of the erect phallus (Frank et al. 1990). For all natal animals, we estimated birthdates (±7 days) using methods described earlier (Holekamp et al. 1996), and for other individuals, we estimated age (±6 months) from tooth wear (Van Horn et al. 2003).

Spotted hyenas exhibit male dispersal, but natal males often delay dispersing from their natal clan for up to several years after reaching sexual maturity at 24 months of age. A clan therefore contains three classes of adults: natal females, immigrant males, and adult natal males that have not yet dispersed (Holekamp & Smale 1998). All natal animals outrank all immigrants (Holekamp & Smale 1998), and among immigrants, the longest-tenured immigrant holds the highest rank and the shortest-tenured immigrant holds the lowest (Smale et al. 1997; East & Hofer 2001). We therefore used an immigrant's arrival date in the clan to determine his social rank, and used the outcomes of dyadic agonistic interactions to confirm this rank order (Holekamp & Smale 1993; Smale et al. 1993).

Behavioral data collection

We observed the hyenas from our vehicles for several hours every morning and evening. When we encountered a subgroup of one or more hyenas, we initiated an observation session by recording the identity and activity of each hyena present; all hyenas within 200m of the subgroup were considered part of the session (Smith et al. 2008). We used all-occurrence sampling

(Altmann 1974) to record a suite of critical incidents, including all aggressive acts and subsequent responses. We limited our analyses to sessions lasting ≥15 minutes, and to individual hyenas that were observed in at least 10 sessions. Each aggressive act was a discrete behavior that was assigned to one of three intensity levels: high intensity aggressive acts were bites and bite-shakes; medium intensity acts were chases and lunges; low intensity acts were pushes, stand-overs, "points" (aggressive posturing), head waves, and displacements (Smith et al. 2007). We defined "dyadic aggression" as aggression that occurred between a single aggressor and a single recipient, and only dyadic aggressive interactions were considered in these analyses. Finally, because we were interested in intrasexual aggression, the aggression we considered here was directed exclusively at individuals of the same sex as the aggressor.

Terminology

When recording an aggressive act, we documented the identity (ID) of the aggressor, the ID of the recipient, and the intensity level of the act. For each aggressor and recipient, we recorded its status in the clan (adult female, adult natal male, or immigrant male), and for immigrant males, we recorded its tenure in the clan. Females were considered adults when they reached 36 months of age or at their date of first conception, whichever occurred first (Smith et al. 2007). Natal males were considered adults when they reached 24 months of age, and all immigrant males were considered adults. For one model, immigrant males were further classified with a binary tenure as either "new immigrants" (tenure <90 days) or "established immigrants" (tenure ≥90 days).

We recorded the conditions of each session as a series of binary categorical variables: "food presence" indicated that at least one food item was present during the session; "adult female presence” indicated that at least one adult female was present during the session; “female near conception [FNC] presence” indicated whether or not there was at least one adult female present in the session who was within ±2 weeks of the date on which we estimated her to have conceived a known litter. These conception dates were calculated based on the known accuracy of our birthdate estimates (±7 days) and the known gestation period in this species of 110 days (Holekamp et al. 1996). We used a 2-week window around the estimated date of conception to incorporate the uncertainty regarding both the birthdate estimate and the estrus period in this species, as the exact estrus cycle remains unknown.

The tight social control imposed by a stable dominance hierarchy means that spotted hyenas almost exclusively direct aggression down the hierarchy, rather than up (Smith et al. 2010; Van Meter 2010). As a result, although an individual may be in a session with multiple conspecifics, effectively, the only potential targets of its aggression are those animals that are lower-ranking than that individual. We therefore controlled for the number of opportunities available to an individual to behave aggressively given the social constraints of spotted hyena society by limiting our analyses to aggression directed at lower-ranking recipients. We quantified the number of opportunities by calculating how many possible “targets” were present with the focal individual in a given session, with a “target” being any lower-ranking individual of the same sex. In our analyses of aggressive acts emitted by each focal individual, we included only individuals with at least one target present. Similarly, when analyzing the aggressive acts received by each focal individual, we needed to control for the number of higher-ranking individuals of the same sex present in the session, as this reflects the number of opportunities the focal individual had to be attacked. We termed these higher-ranking individuals “potential aggressors,” and only included focal individuals with at least one potential aggressor in our analyses of aggressive acts received by each focal individual.

Statistical Analyses

To compare intrasexual aggression rates among adult females, immigrant males, and adult natal males, we first calculated individual aggression rates at each level of aggression intensity. To do this, we divided the number of dyadic intrasexual aggressive acts exhibited by an individual in a given session by the number of potential dyadic targets present in that session, and then again by the length of the session in hours. We obtained lifetime aggression rates for each individual at each intensity level by taking the mean aggression rate at that intensity for all sessions in which that individual was observed. These lifetime aggression rates were subsequently used as the unit of replication in our descriptive comparison of aggressive acts exhibited by females, immigrants, and adult natal males.

To test our hypotheses regarding the function of male-male aggression in this species, we ran a series of Bayesian Markov Chain Monte Carlo generalized linear mixed models using the R package MCMCglmm (Hadfield 2010). Our first model, model_{AE}, was designed to test the rate predictions of both the first hypothesis, which suggested that males should exhibit more aggressive acts in the presence of females, and the third hypothesis, which posited that males should exhibit more aggressive acts in the presence of food. We therefore modeled the number of aggressive acts a male exhibited in a session; however, unlike our calculations of lifetime aggression rates, here we modeled the absolute number of aggressive acts a male exhibited, without dividing by the session length or number of possible targets available to the male in the session. Instead, we incorporated both of these other variables as fixed effects in the model, using the log of session length as an offset term. Model_{AE} then also included the following fixed effects: the dispersal status of the male (immigrant vs. adult natal), food presence, food presence x status, adult female presence, FNC presence, FNC presence x status, tenure in the clan, and tenure x status. We initially also included female presence x status, but this term introduced high levels of multicollinearity, it was not significant in the model, and removing it did not affect our conclusions about any of the other effects, so we removed it. For each categorical fixed effect (status, female presence, FNC presence, and food presence), we set the reference level of the model at the value with the highest sample size. The reference level of model_{AE} was therefore an immigrant male in the presence of at least one female, but in the absence of an FNC or food; all posterior mean estimates of the coefficients for each parameter are presented relative to the reference level (for this model and all subsequent models). Finally, because multiple hyenas were present in most sessions and individual hyenas appeared in multiple sessions, aggressor ID and session number were both included as random effects. We standardized tenure to have a zero mean and unit variance before including it in the model; all other subsequent standardizations were done similarly.

Our second model, model_{AR}, was designed to test the rate prediction of the second hypothesis, which was that if males utilize aggression to restrict clan membership, new immigrants should receive more aggression than should established immigrants. We therefore modeled the number of aggressive acts a male received in a session, rather than the number of acts he directed at others. Because we were only interested in testing the effect of being an established vs. new immigrant, model_{AR} was limited only to immigrant male recipients of aggression. In addition, we wanted to distinguish between aggression received from other immigrants and aggression received from adult natal males, so when an immigrant was present in a session with potential aggressors from both categories, the counts of aggressive acts he received from males in each status category were included as separate data points in the model. Thus, the fixed effects were the binary tenure of the recipient (established immigrant vs. new immigrant), the dispersal status of the aggressor(s) (adult natal vs. immigrant), and recipient binary tenure x aggressor(s) status. We also included the number of potential aggressors present with the male in the session as a fixed effect (standardized prior to inclusion in the model), and the log of session length as an offset term. Once again, for each categorical fixed effect (binary tenure of the recipient and aggressor status), we set the reference level of the model at the value with the highest sample size. The reference level of model$_{AR}$ was therefore an established immigrant receiving aggression from another immigrant. Recipient ID and session number were included as random effects.

Our third model, model$_{I}$, tested predictions of all three hypotheses regarding the intensity of aggression. In contrast to the previous models, in which the response variable was a count of aggressive acts exhibited or received (which could be null), this model simply assessed whether aggressive acts that did occur were of high, medium, or low intensity. Thus, the response variable in model$_{I}$ was ordinal, and each data point was a distinct aggressive act. Model$_{I}$ included the following fixed effects: food presence, female presence, FNC presence, and a dummy categorical variable representing the five possible combinations of aggressor status (immigrant vs. adult natal) and recipient status (new immigrant, established immigrant, and adult natal); we could not simply include an interaction effect between aggressor status and recipient status because immigrants never aggress against adult natal males, so an interaction effect would have been inappropriate. In addition, we included a binary fixed effect indicating whether or not an attack had occurred within the same aggressor/recipient pair in the previous 5 minutes. This term was included to control for the possibility that an aggressor may be more likely to increase the intensity with which he aggresses against a specific recipient if he has aggressed against that recipient in the very recent past. The interaction effects of food presence x status, female presence x status, or FNC presence x status introduced high levels of multicollinearity, primarily because adult natal males exhibited so few aggressive acts, so we excluded them. As in the previous two models, for each categorical fixed effect (aggressor status, recipient status, female presence, FNC presence, food presence, and aggression in previous 5 minutes), we set the reference level of the model at the value with the highest sample size. The reference level of model$_I$ was therefore an established immigrant receiving aggression from another immigrant in the presence of a female, but in the absence of an FNC or food, and without an aggression having occurred in the previous 5 minutes within the same aggressor/recipient pair. Session number, aggressor ID, and recipient ID were included as random effects.

For all fixed effects, we report the posterior mean estimates relative to the intercept (which represents the reference level for all categorical variables, and the mean for all standardized continuous variables), the 95% credible intervals (CI), and the corresponding p-values. We deemed a fixed effect significant if its 95% CI did not overlap with zero. We report the proportion of variance explained by each random effect relative to the sum of the total variance, expressed as a percent. For details on the model specifications, including the priors and convergence diagnostics, see Appendix A. All statistical analyses were carried out in R v. 2.13.0 (R Development Core Team 2011).

Results

To calculate rates of intrasexual aggression, we analyzed data from 99 adult females, 56 immigrant males, and 72 adult natal males. The lifetime intrasexual aggression rates among both immigrant males and adult natal males were comparable with the lifetime aggression rates among adult females (Figure 1.1), particularly at low and medium intensities. Variation in aggression rates at low and medium intensities was greater among immigrant males than among adult females or adult natal males.

Our first model, model_{AE}, examined 4276 sessions in which there were at least two adult males present, so that there was the potential for one male to aggress against another. 56 immigrants and 72 adult natal males appeared as potential aggressors in these sessions (i.e. they may not have exhibited any aggression, but they did have at least one potential target). The complete results of each model, including posterior mean estimates for each fixed effect with corresponding credible intervals and p-values, are shown in Table 1.1. Because model_{AE} used a log-link function, the reported coefficients are log-transformed. Therefore, in order to interpret the absolute values of the coefficients, one must take the inverse of the natural log of the reported value. For example, the intercept indicates that an immigrant male with the mean tenure and the mean number of targets, in the presence of an adult female but not in the presence of an FNC or food, would be expected to exhibit 0.013 aggressive acts per hour. One could perform similar back transformations for other covariates in the model, or for the coefficients in model_{AR}.

Males aggressed significantly more in the absence of at least one adult female than when no female was present. There was no significant effect of the presence of a female near conception on the number of aggressive acts a male exhibited, regardless of the male’s status (immigrant vs. adult natal male). There was a significant interaction effect between the presence of food and the status of the individual exhibiting aggression (Figure 1.2). When food was not present, immigrant males aggressed significantly more often than did adult natal males. Both immigrants and adult natal males aggressed significantly more often in the presence of food than when food was absent, and in the presence of food, there was no difference in aggression rates between immigrants and adult natal males. Among immigrant males, aggression rates significantly increased with tenure in the clan. Unsurprisingly, the number of targets present was positively correlated with the number of aggressive acts a male exhibited during a session. Hyena ID accounted for 7.63% of the total variance, and session accounted for 49.76%.

The second model, model_{AR} (Table 1.1), included 4088 sessions in which there were at least two adult males present, so that the potential existed for one individual to receive aggression. 65 immigrants appeared as potential recipients in these sessions. There was a significant interaction effect between the recipient's binary tenure and the status of the aggressor(s) (Figure 1.3): established immigrants received significantly less aggression from immigrant males than did new immigrants, but there was no difference between established and new immigrants in the aggression received from adult natal males. The number of potential aggressors present was positively correlated with the number of aggressive acts a male received in a session. Hyena ID accounted for 2.12% of the total variance, and session accounted for 57.93%.

The third model, model_{I} (Table 1.1), contained 1677 dyadic aggressive interactions involving 107 different aggressors and 85 different recipients, occurring over the course of 726 sessions. Because model_{I} used a logit-link function, the reported coefficients are logit-transformed. Therefore, in order to interpret the absolute values of the coefficients, one must take the inverse logit of the reported value, similar to the required transformations described in the results of model$_{AE}$. The likelihood that an aggressive act would be of high intensity when directed by an immigrant male toward another immigrant was not affected by the recipient's binary tenure (established vs. new). Similarly, aggressive acts directed by adult natal males toward established immigrants and toward new immigrants were equally likely to be of high intensity, but aggression directed by adult natal males toward other adult natal males was significantly more likely to be of high intensity than were aggressive acts within all other status combinations of aggressor-recipient dyads. Neither the presence of an adult female nor the presence of a female near conception had an effect on intensity, but the presence of food significantly increased the likelihood that aggression would be of high intensity. Aggression was significantly more likely to be of high intensity if a previous aggressive act had occurred within the same dyad within the last five minutes. Finally, aggressor ID accounted for 8.66% of the total variance, recipient ID accounted for 6.57%, and session accounted for 41.60%.

Discussion

Our finding that immigrant males were equally likely to exhibit aggression as were adult females (Figure 1.1) is in contrast to previous assumptions that male hyenas are less aggressive than females (e.g., East & Hofer 2001). This misconception is almost certainly due to the fact that the absolute aggression rates do differ when one does not control for the number of potential targets, as originally demonstrated by Van Meter (2010). The female dominance characteristic of this species means that immigrant males typically have far fewer potential targets than do adult females. We chose to control for this in our analysis of lifetime aggression rates because we were interested in comparing rates of intrasexual aggression among females, immigrant males, and adult natal males within the bounds of their social constraints, rather than in an absolute sense.

We did not find support for the hypothesis proposing that male-male aggression functions to prevent other males from accessing females. Males were actually less aggressive to one another in the presence of a female than when females were absent, suggesting that if males are competing for access to females, the mechanism is less direct than simply jockeying for positions in close proximity to females. In fact, the negative relationship we found between male-male aggression and female presence, coupled with the high degree of female choice in this species, raises the possibility that female hyenas actually prefer more docile males, as has been shown in some primates (Chapais 1983; Richard 1992; Bercovitch 1997), or else females prefer a social environment generally lacking in aggression, regardless of the participants in the conflict.

Our data supported the hypothesis that males use intrasexual aggression to restrict group membership by deterring prospective immigrants trying to join the clan (Figure 1.3). Immigrants still in the tenuous first three months of their tenure received significantly more aggression from other immigrants than did established immigrants. In contrast, adult natal males did not seem to make this distinction, and generally aggressed less toward immigrants than did other immigrants. These results reinforce the idea that males use aggression to compete indirectly for access to females because adult natal males will soon be dispersing out of the clan, and therefore have little to gain by controlling the number of potential mates available to females in their natal clan (Packer 1979a). In contrast, immigrant males benefit from limiting the total number of immigrants in the clan because resident immigrants typically sire the vast majority of cubs (Engh et al. 2002), so restricting immigration reduces the amount of male-male competition (Mitani et al. 1996).

Both immigrant and adult natal males displayed more frequent and more intense aggression in the presence of food than when food was absent (Figure 1.2), supporting our third hypothesis that males utilize intrasexual aggression to access food. The baseline aggression rate of adult natal males was lower than that of immigrant males, but this difference disappeared when food was present. The contrast between these results and the results from tests of our second hypothesis demonstrate that, whereas access to females may be a more valuable commodity to immigrants than to adult natal males, food appears to be equally valuable to both immigrants and adult natal males.

The coupling of monomorphism in body size with competition over mates might indicate that actual fighting does not improve reproductive success (Richard 1992), as East et al. (2001) suggested. Kappeler (1990) proposed that in male prosimians, which compete physically through arboreal chases much more than through actual combat, intrasexual competition might select for speed and agility rather than weaponry. The same could be true in spotted hyenas, for the vast majority of aggressive interactions analyzed here did not involve any physical contact. This could be a contributing factor to the female-biased size dimorphism in this species (Swanson et al. 2011).

Richard (1992) argued that all male-male aggression can be viewed as competition for access to mates. How direct the link is between the competition (aggression) and the resource (mates) certainly varies, however, and appears substantially less direct in role-reversed species, like the spotted hyena, than in non-role-reversed species. The data presented here, showing that males are less aggressive in the presence of females and that immigrants use aggression to restrict clan membership, support the hypothesis that males compete for access to mates via an endurance rivalry, rather than through overt combat (Richard 1992; Andersson 1994; East & Hofer 2001).

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Figure 1.1. The hourly number of intrasexual aggressive acts exhibited by individual adult female, immigrant male, and adult natal male spotted hyenas (*Crocuta crocuta*) at three levels of aggression intensity. These rates represent the number of aggressive acts an individual exhibited per potential target, so they control for the number of opportunities an individual realistically had to aggress given the social constraints imposed by the dominance hierarchy. Horizontal lines indicate medians, box edges show 1st and 3rd interquartile ranges (IQR), and whiskers represent closest data points within (1.5 x IQR) of box edges. Sample sizes represent individuals; we calculated a separate lifetime mean hourly rate for each individual at each intensity level, so the same set of individuals was assessed at each level.

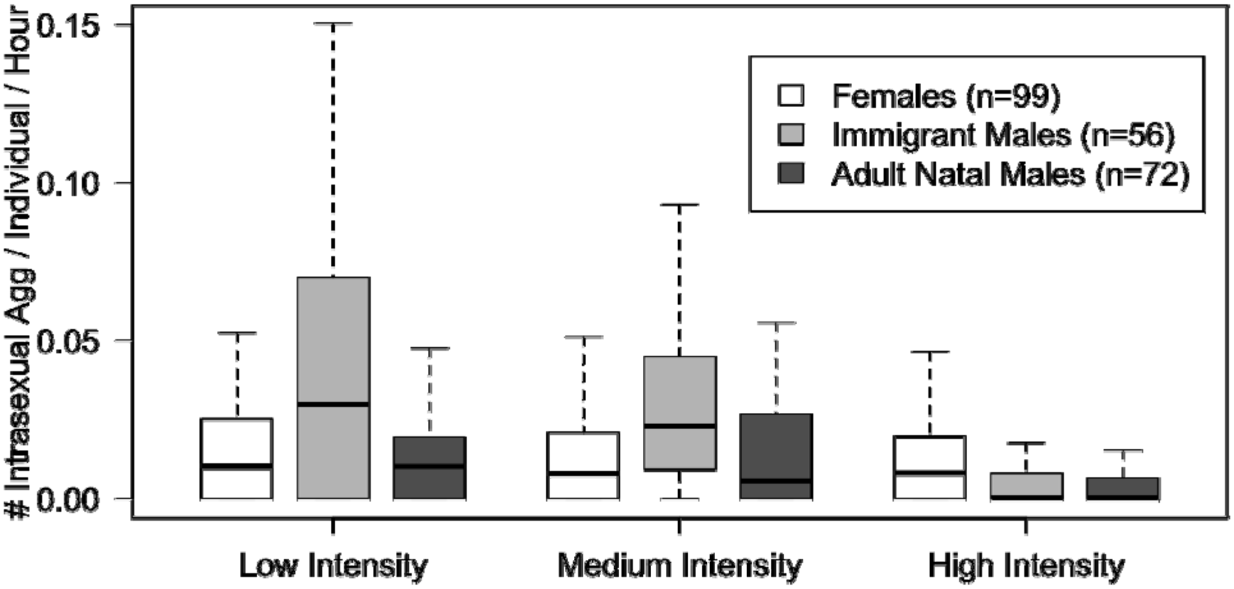

Figure 1.2. The number of aggressive acts male spotted hyenas (*Crocuta crocuta*) exhibited per hour as a function of both his status in the clan (immigrant or adult natal male) and whether or not food was present in the session. Posterior mean estimates of the number of aggressions are presented with error bars representing the 95% credible intervals. Error bars labeled with the same letter indicate that those posterior means were not significantly different. These results correspond with those from model$_{AE}$ (Table 1.1).

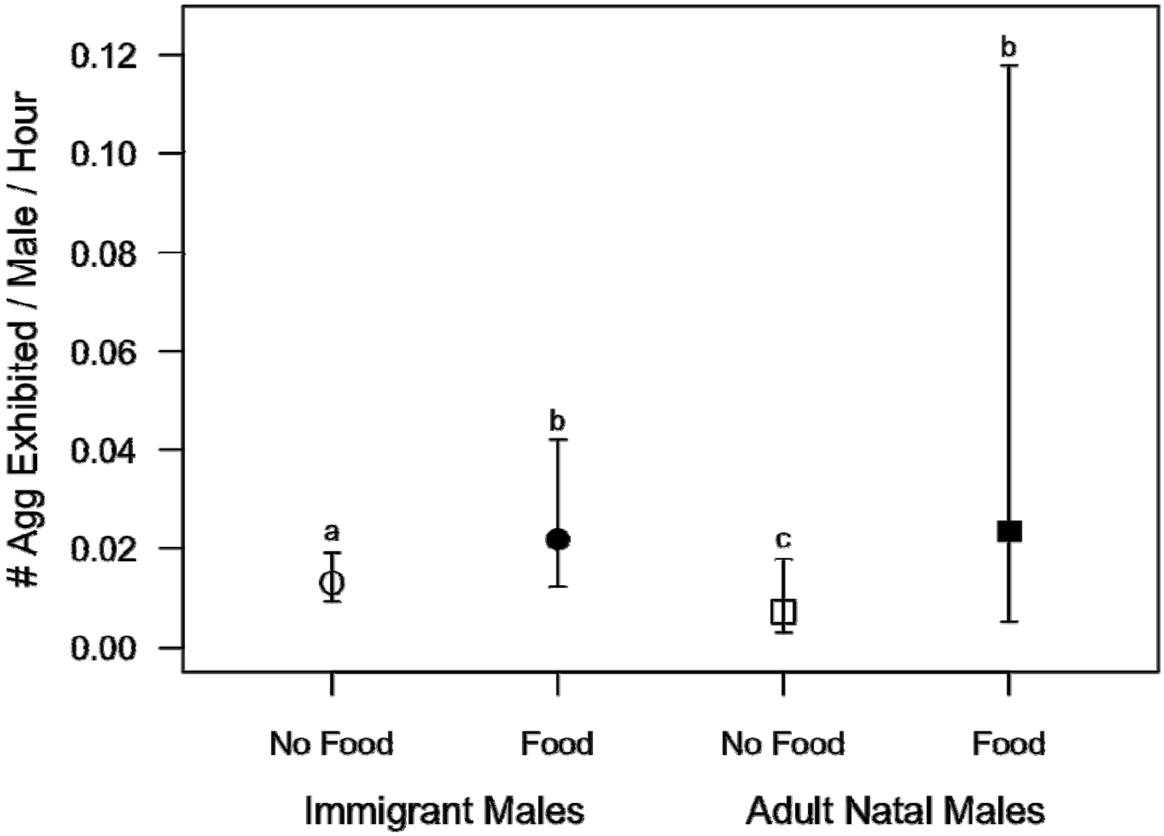

Figure 1.3. The number of aggressive acts immigrant male spotted hyenas (*Crocuta crocuta*) received per hour from other immigrant males and from adult natal males, as a function of the recipient's binary tenure in the clan (established immigrants: tenure ≥ 90 days; new immigrants: tenure < 90 days). Posterior mean estimates of the number of aggressions are presented with error bars representing the 95% credible intervals. Error bars labeled with the same letter indicate that those posterior means were not significantly different. These results correspond with those from model$_{AR}$ (Table 1.1).

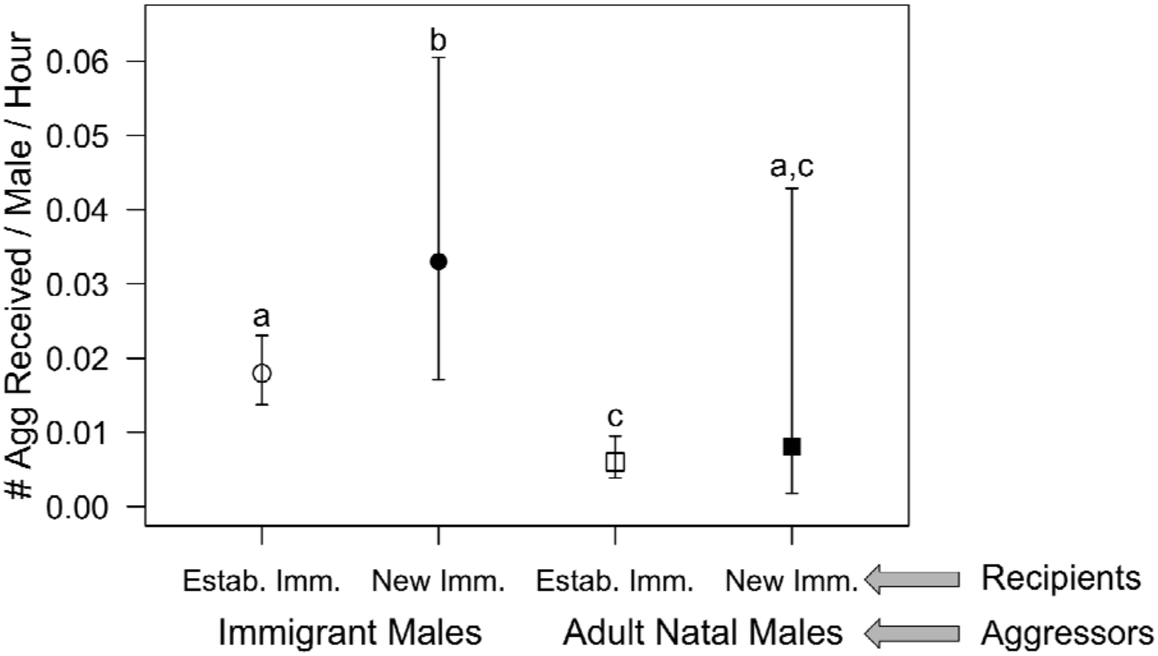

Table 1.1. Estimates of Bayesian mixed models using MCMCglmm explaining the number of aggressive acts a male spotted hyena (*Crocuta crocuta*) exhibited (model$_{AE}$) or received (model$_{AR}$), and the intensity of such acts (model$_{I}$). Posterior mean estimates of fixed effect coefficients are reported with the 95% credible intervals (CI) and pMCMC values. For model$_{AE}$ and model$_{AR}$ the posterior mean estimates are the log-transformed values of the coefficients and represent the hourly aggression rates relative to the intercept. For model$_{I}$ positive coefficients indicate a higher likelihood that an aggressive act would be of high intensity. For all models, significant effects are presented in bold, and random effects are presented as the percent of the total variance they explained. Continuous covariates were standardized prior to their inclusion in the model.

Model	Sample size	Parameter	Posterior mean (95% CI)	*p*MCMC
model$_{AE}$	128 hyenas,	Intercept (Immigrants; Female Present; No FNC Present; No Food Present)	-4.341 (-4.684, -3.957)	-
	4276 sessions	**Adult Natal Males (ANMs)**	**-0.589 (-1.100, -0.070)**	**0.036**
		No Female Present	**0.581 (0.064, 1.050)**	**0.016**
		Intxn between Status and FNC Presence:		
		Immigrants: FNC Present	-0.045 (-0.323, 0.220)	0.752
		ANMs: FNC Present	-0.240 (-0.656, 0.174)	0.276
		Intxn between Status and Food Presence:		
		Immigrants: Food Present	**0.515 (0.276, 0.788)**	**<0.001**
		ANMs: Food Present	**0.665 (0.253, 1.100)**	**<0.001**
		Immigrant Tenure	**0.373 (0.208, 0.499)**	**<0.001**
		Number of Targets	**0.374 (0.256, 0.478)**	**<0.001**
		Random effects:	Percent of Variance:	
		Hyena ID	7.635	-
		Session	49.759	-

Table 1.1 (cont'd)

model_{AR}	65 hyenas,	Intercept (Aggressors: Immigrants; Recipients: Established Immigrants)	-4.020 (-4.288, -3.771)	-
	4088 sessions	**Agg: Immigrants; Recip: New Immigrants**	**0.609 (0.218, 0.967)**	**0.002**
		Agg: ANMs; Recip: Established Immigrants	**-1.094 (-1.271, -0.886)**	**<0.001**
		Agg: ANMs; Recip: New Immigrants	-0.311 (-1.010, 0.541)	0.440
		Number of Potential Aggressors	**0.207 (0.119, 0.286)**	**<0.001**
		Random effects:	Percent of Variance:	
		Hyena ID	2.506	-
model_{I}	1677 aggressions,	Intercept (Agg: Immigrants; Recip: Established Immigrants; Female Present; No FNC Present; No Food Present; No Agg in Previous 5 Minutes)	-0.466 (-0.779, -0.130)	-
	107 aggressors,	Intxn between Agg Status and Recip Binary Tenure:		
	85 recipients,	Agg: Immigrants; Recip: New Immigrants	-0.234 (-0.677, 0.208)	0.314
	726 sessions	Agg: ANMs; Recip: Established Immigrants	0.219 (-0.185, 0.611)	0.266
		Agg: ANMs; Recip: New Immigrants	0.883 (-0.292, 2.033)	0.130
		Agg: ANMs; Recip: ANMs	**0.730 (0.153, 1.281)**	**0.024**
		No Female Present	-0.088 (-0.589, 0.450)	0.734
		FNC Present	0.170 (-0.107, 0.449)	0.224
		Food Present	**0.329 (0.081, 0.603)**	**0.018**
		Aggression Between Agg/Recip Pair in Previous 5 Minutes	**0.451 (0.222, 0.660)**	**<0.001**
		Random effects:	Percent of Variance:	
		Aggressor ID	8.66	-
		Recipient ID	6.57	-
		Session	41.60	-

APPENDIX

APPENDIX A

We ran all MCMCglmm (Hadfield 2010) models such that 1000 posterior samples were kept; these samples comprised the posterior distribution for each model and were used to generate all posterior estimates. Models were run until they reached acceptably low levels of autocorrelation (<0.2 for successive iterations) for both the fixed effects and variance components (Plummer et al. 2006; Hadfield 2010) and until they reached proper convergence, which we assessed visually using the trace plots for both the fixed effects and variance components, and computationally using Geweke's convergence diagnostic (Plummer et al. 2006). As such, the total number of iterations differed for each model: model_{AE} and model_{AR} were both run for 600,000 iterations, with a burn-in period of 100,000, a thinning interval of 500, and a poisson distribution; model_{I} was run for 800,000 iterations, with a burn-in period of 200,000, a thinning interval of 600, and an ordinal distribution. For all models, we ran three parallel (i.e., independent but identical) MCMC chains and checked convergence among these chains using the potential scale reduction factors (also known as the Gelman-Rubin statistic; Gelman & Rubin 1992). The first of these chains was then used to report posterior distributions and estimates for the model parameters. We used variance inflation factors and condition indices to assess the multicollinearity of our models (Heiberger 2009; Hendrickx 2010). All statistical analyses were carried out in R v. 2.13.0 (R Development Core Team 2011).

The Bayesian nature of MCMCglmm requires the specification of priors for each model. The priors for fixed effects have two parameters, the mean (mu) and the variance (V); MCMCglmm uses a normal prior that is centered around zero and has a large variance (Hadfield 2010). For random effects, MCMCglmm uses an inverse-Wishart prior, which generally has two parameters, the variance (V) and the degree of belief in that variance (nu). For model$_{\mathrm{AE}}$ and model$_{\mathrm{AR}}$, we used mu=0 and V=1e+6 for the fixed effects, with the exception of the offset term, for which we used mu=1 and V=1e-6. For the random effects and residual variance, we used V=1 and nu=0.002. For model$_{\mathrm{I}}$, we used mu=0 and V=1e+8 for the fixed effects. For the random effects, we used V=1 and nu=1, and for the residual variance, we used a fixed prior of V=1, because MCMCglmm cannot estimate residual variance for ordinal data. However, for all three models, we tried a variety of priors and found the model results to be insensitive to changes in the prior specifications.

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CHAPTER 2
EJACULATE QUALITY IN SPOTTED HYENAS: INTRASPECIFIC VARIATION IN RELATION TO LIFE HISTORY TRAITS

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Abstract

Sperm competition has received much attention in recent years as a primary form of intrasexual competition among males, but little is known about variation in ejaculate quality in natural mammal populations, particularly among carnivores. Here, we documented variation in semen characteristics among wild male spotted hyenas (*Crocuta crocuta*). We then tested hypotheses suggesting that reproductive senescence among aging male hyenas is reflected in declining ejaculate quality, and that dispersal status affects ejaculate quality. To address these questions, we electroejaculated 20 wild males, including both immigrants and adult natal males. We found no support for the hypothesis that male spotted hyenas experience reproductive senescence with respect to their ejaculate quality, but we did find that immigrant males had significantly higher ejaculate quality than adult natal males, suggesting that adult natal males may experience reproductive suppression before dispersing. Finally, to test the assumption that an individual's ejaculate quality is consistent over time, we obtained repeated samples from 6 captive male hyenas. This captive work demonstrated significant individual repeatability in ejaculate quality and illustrated the utility of complementing field research with laboratory study. By exploring variation in ejaculate quality and its relationship with life history traits in this unique species, we have highlighted new avenues for potential research regarding how sexual selection manifests in sex role-reversed species.

Keywords: *Crocuta crocuta*, dispersal status, ejaculate quality, individual repeatability, intraspecific variation, sperm competition, spotted hyena

Introduction

Sexual selection theory predicts that when females have the limiting reproductive rate, males should compete for access to those females (Darwin 1871; Andersson 1994). This competition is not over access to copulations with females, but rather over access to female gametes (Parker 1970). Thus, in many species male-male competition continues after copulation via sperm competition, which occurs when a female copulates with multiple males and the ensuing rival ejaculates compete to fertilize her ova (Birkhead & Pizzari 2002; Birkhead et al. 2008; Pitnick & Hosken 2010). Since it was first proposed as a mechanism of selection (Parker 1970), sperm competition has been shown to affect numerous ejaculate traits, including ejaculate volume, sperm concentration, number of sperm, sperm motility, and sperm length (Malo et al. 2005; Birkhead et al. 2008; Parker & Pizzari 2010; Montoto et al. 2011; Tourmente et al. 2011).

Sperm competition is thought to be widespread in mammals (Gomendio et al. 1998; Dixson & Anderson 2004; Stockley 2004). Considerable progress has been made in recent years toward understanding the role of sperm competition in mammalian sexual selection and its effects on ejaculate traits (e.g., Schulte-Hostedde & Millar 2004; Gomendio et al. 2007; Ramm & Stockley 2008; Firman & Simmons 2011; Montoto et al. 2011; Tourmente et al. 2011). However, most research on mammalian sperm has come from captive or laboratory populations, with only rare exceptions (e.g., Gomendio et al. 2007; Bonanno & Schulte-Hostedde 2009), so relatively little information exists on variation in ejaculate quality in natural populations. Even less is known about ejaculates in free-living carnivore populations, presumably due to the challenges of sampling these species in nature. Most semen samples collected from carnivores have been obtained from captive animals (see Appendix B; for exceptions, see Brown et al. 1991; Morato et al. 2001; Crosier et al. 2007; Gañán et al. 2010), so sperm competition in carnivores remains poorly understood. Iossa et al. (2008) made significant progress by investigating sperm competition across carnivores as a function of various life history parameters, using relative testes size (RTS) as an indicator of the intensity of sperm competition (Gage & Freckleton 2003; Soulsbury 2010). However, testing many predictions of sperm competition theory requires collection of ejaculate samples from free-living males, particularly males for whom paternity data are also available (e.g., Gomendio et al. 2007). To our knowledge, such comprehensive data do not currently exist for any carnivore species.

Here we took the first step toward closing this gap by sampling ejaculates in wild spotted hyenas (*Crocuta crocuta*) for which data on paternity success are also being collected. Several lines of evidence suggest that sperm competition operates as an important selective force in this species. First, female spotted hyenas are polyandrous, and approximately one-third of twin litters have multiple sires (Engh et al. 2002; East et al. 2003). Multiple paternity implies that, at the moment of fertilization, multiple males are still competing via sperm competition (Gomendio et al. 1998). In fact, multiple paternity has been shown in other species to correlate with the presence of sperm competition (Ramm et al. 2005; Dean et al. 2006; Soulsbury 2010).

Second, the reproductive tract of the female spotted hyena is unusually long and convoluted (Cunha et al. 2003), which lengthens the sperm's path to the ova far beyond that of other comparably-sized mammals. Although sperm are deposited above the penile clitoris through which copulation occurs, they must then circumnavigate the many dead ends in the coiled vaginal lumina (Cunha et al. 2003). The length and complexity of the reproductive tract in female spotted hyenas should theoretically strengthen selection on ejaculate traits (Eberhard 1996; Gomendio et al. 1998; Birkhead et al. 2005), and therefore intensify sperm competition.

Third, theory predicts there should be intense competition among male spotted hyenas because females have the limiting reproductive rate (Kruuk 1972; Holekamp et al. 1996), and males show no parental care (Kruuk 1972) but considerable reproductive skew (Engh et al. 2002; Holekamp et al. 2012). Despite this prediction, male spotted hyenas engage in less intense physical combat than that typical of other male mammals (Kruuk 1972; Goymann et al. 2003), indicating that males must compete via alternative mechanisms, one of which may be sperm competition (Andersson 1994).

However, two lines of evidence oppose the hypothesis that sperm competition is an important selective force in spotted hyenas. First, intensity of sperm competition is positively correlated with RTS in many taxa, including mammals (Harcourt et al. 1981; Kenagy & Trombulak 1986; Gage 1994; Gomendio et al. 1998; Gage & Freckleton 2003; Ramm et al. 2005; Soulsbury 2010), and experimental manipulations of sperm competition have demonstrated this relationship to be causal (Hosken et al. 2001; Hosken & Ward 2001; Pitnick et al. 2001). Therefore, if male spotted hyenas are indeed competing intrasexually via sperm competition, then male hyenas should theoretically have large testes relative to their body size (Dixson & Anderson 2004; Iossa et al. 2008; Soulsbury 2010). Instead, the observed RTS in spotted hyenas aligns more closely with that of monandrous species (Soulsbury 2010). Second, Iossa et al. (2008) demonstrated that sperm competition is more prevalent in species with synchronous female estrus cycles, which is not the case in spotted hyenas (Holekamp et al. 1999).

These seemingly contradictory features make spotted hyenas a fascinating species in which to investigate variation in ejaculate quality in a natural population. Our first goal here was therefore to describe ejaculate characteristics in this species and to establish the degree of intraspecific variation in ejaculate quality. Additionally, to verify that any variation we found in ejaculate quality was indicative of true between-individual differences and not merely due to within-individual variability (Tregenza et al. 2009), we examined repeatability in the quality of ejaculates obtained repeatedly from a small group of captive male hyenas.

Our second goal was to test specific hypotheses regarding ejaculate quality among male spotted hyenas. East et al. (2003) hypothesized that the surprisingly low reproductive success of the highest-ranking male spotted hyenas, which are also often the oldest males present (Engh et al. 2002; East et al. 2003), might be due to reproductive senescence in ejaculate quality. The paternal age effect, in which male reproductive fitness decreases at advanced ages (Stene et al. 1977), manifests in semen quality in many mammals (Wang et al. 1993; Wolf et al. 2000; Kidd et al. 2001; Thongtip et al. 2008). Paternal age effects in carnivore ejaculates are less clear, perhaps because very old males are rarely sampled. However, if reproductive senescence is occurring in spotted hyenas, then ejaculate quality should be lower among the oldest wild males (10+ years) than among males sampled during their reproductive prime (5-10 years).

Finally, we tested the hypothesis that dispersal status affects ejaculate quality. Male spotted hyenas reach puberty at approximately 24 months of age (Matthews 1939; Glickman et al. 1992) and they then disperse from their natal clans between 2 and 5 years of age (Smale et al. 1997; Van Horn et al. 2003; Boydston et al. 2005). Every spotted hyena clan therefore contains both adult immigrant males and adult natal males that are post-pubertal but have not yet dispersed (Holekamp & Smale 1998). Although both these groups of males represent potential mates for females, immigrant males sire over 97% of cubs (Engh et al. 2002), so dispersal status has a strong effect on a male’s reproductive success (Van Horn et al. 2008). It is possible that adult natal males experience reproductive suppression (Garber et al. 1996; Holekamp & Sisk

2003), but it is unknown whether this suppression would affect ejaculate quality. Reproductive suppression in other species has been shown to reduce testes mass, but its influence on sperm quality is unclear (Faulkes et al. 1994; Fitzpatrick et al. 2005). If dispersal status does affect ejaculate quality in spotted hyenas, then immigrant males should have higher quality semen than adult natal males.

Methods

Subject animals

Our wild study population was comprised of males from 3 clans of spotted hyenas continually monitored in the Masai Mara National Reserve, Kenya. Spotted hyenas breed year round, but all wild males were sampled between August and December in 2008 and 2009. We could identify every individual based on unique spots and other markings (Frank 1986), and we could visually determine sex based on the dimorphic morphology of the erect phallus (Frank et al. 1990). Birthdates of natal individuals were estimated (±7 days) based on methods described in Holekamp et al. (1996), and ages of other individuals (±6 months) were based on tooth wear (Van Horn et al. 2003).

Spotted hyena clans contain up to 90 individuals, including adult males, adult females and natal juveniles of both sexes (Frank 1986). Most of the adult males in any clan are immigrants born elsewhere, and these immigrants have near-zero values of genetic relatedness to one another (Van Horn et al. 2004). We classified immigrant males into 3 age classes: young (age < 5 years; $n=5$), intermediate (5 years $\leq$ age < 10 years; $n=7$), and old (10 years $\leq$ age; $n=4$). We called post-pubertal (i.e. >24 months old) natal males that had not yet dispersed adult natal males. Social ranks of immigrant males are determined strictly by their tenure in the clan (Smale et al. 1997; East & Hofer 2001), with the earliest arrival holding the highest rank and the most recent arrival holding the lowest. We therefore deduced an immigrant male's rank based on his date of arrival in the clan, and confirmed this assignment based on outcomes of dyadic agonistic interactions (Holekamp & Smale 1993; Smale et al. 1993).

To examine repeatability in ejaculate characteristics among individual spotted hyenas, we needed to sample the same males multiple times. This was not feasible with wild hyenas, so we utilized 6 captive males housed at the Field Station for Behavioral Research at the University of California, Berkeley, CA. Berger et al. (1992) describe husbandry conditions at this facility. Of these 6 captive males, 4 had been used in hormone manipulation studies that may have affected semen traits, so the descriptive data on captive spotted hyenas that we report in Appendix B were calculated based only on the 2 control males, but all 6 were used in the repeatability analyses. We sampled each male in February 2010 and again in September 2010.

All research followed the guidelines of the American Society of Mammalogists (Sikes & Gannon 2011) and was approved by institutional animal care and use committees at both Michigan State University and the University of California, Berkeley.

Semen collection and evaluation

Each wild hyena was anesthetized prior to semen collection with Telazol (W.A. Butler Co., Brighton, MI, USA, 6.5mg/kg); captive hyenas were anesthetized with a cocktail of xylazine (AnaSed Injection, Akom Inc., Decatur, IL, 1mg/kg) and ketamine (Ketaset Ketamine, Fort Dodge Animal Health, Fort Dodge, IA, 5mg/kg). Once the hyena was fully unconscious, we measured the length and width of the testes using calipers, then inserted a sterile, lubricated catheter (3.3mm diameter) through the urethra to drain urine from the bladder to avoid contaminating the semen. We then rinsed the bladder and urethra by injecting and draining sterile saline through the catheter.

Semen collection was performed using the electroejaculation procedure described by Wildt et al. (1983). Briefly, we first lubricated a rectal probe (2.5cm in diameter, 30cm long; P-T Electronics, Sandy, OR) with ultrasound gel. We then inserted the probe approximately 15-20cm into the anus of the male, targeting the pudendal nerve with the electrodes on the end of the probe. The probe was connected to an electro-stimulation unit that we used to manually administer low-level electric pulses in the following cycle: 3 seconds from zero to peak amperage, 1s at peak amperage, 1s from peak to zero amperage, and 3s at zero amperage before another pulse began. We delivered pulses in sets of 10, with 2 minutes of rest between sets. The amperage of the first set was 50mA, and we gradually increased the amperage over the course of multiple sets to 75mA, 100mA, and 150mA (~4-6V); amperage never exceeded 150mA, voltage never exceeded 6V, and the total number of stimulations an animal received never exceeded 120. We collected any semen in a fresh vial after each set of 10 pulses, and stopped the procedure when the first of the following occurred: the animal lost its erection, the animal stopped producing semen, the total number of stimulations received reached 120, or the effects of the anesthesia began to subside. Each vial was analyzed independently of other vials.

We assessed the following traits at 400x magnification (Wildt et al. 1987): concentration (sperm/mL $\times 10^6$), percent motile, progressive status (average rate of forward progression of motile sperm on a scale of 0-5, as a proxy for velocity), total sperm length (μm), sperm midpiece length (μm), sperm head length (μm), and sperm motility index, calculated as (percent motile + [20 x progressive status])/2. We also measured the volume (ml) of ejaculate in each vial, and used this to calculate total ejaculate volume (ml) and total number of sperm per ejaculate. All vials were kept shaded throughout and after the procedure. Due to logistic constraints, we were unable to assess morphological normality of sperm. Percent motile and progressive status were both evaluated within 10-15 minutes of semen collection. We then diluted 50μl aliquots of semen with 1ml of deionized water to immobilize the sperm so we could assess sperm concentration. We used an ocular micrometer to measure the length of multiple unbent sperm from each male, including the length of the head and midpiece; how many we measured depended on our ability to locate unbent sperm (range = 2-8; mean = 5). For the captive hyenas, all the same measurements were collected with the exception of the sperm lengths (total, midpiece, and head), which were not measured due to equipment limitations.

Statistical analyses

We calculated the median value for each trait within each individual across semen vials, then used these median values to calculate means for each trait among either immigrant males (n=16) or adult natal males (n=4). By using the median values, we hoped to control for any effects minor variation in the electroejaculation procedure may have had on ejaculate traits. To explore relationships among the various sperm traits, we performed a principal components analysis (PCA) on median values of 4 of the primary sperm traits: concentration, percent motile, progressive status, and sperm length. Before performing the PCA, we assessed the normality of each dataset using the Shapiro-Wilk test and then transformed the input variables to reduce the influence of outliers, achieve normality, and standardize proportional data. We also standardized the variables prior to performing the PCA.

We assessed individual repeatability among captive hyenas using 2 different measures of consistency. First, we calculated the Spearman rank correlation coefficients (R_S) for each trait.

We also evaluated individual repeatability using intra-class correlation coefficients (ICC: Shrout & Fleiss 1979), with values closer to 1 indicating high consistency, and values closer to 0 indicating little or no consistency.

To test for significant relationships between testes size and ejaculate quality, we used linear models after making the appropriate transformations. We used absolute testes size, rather than RTS, because body mass in spotted hyena can fluctuate greatly over short periods of time and is not necessarily an accurate reflection of overall body size (Swanson et al. 2011). To test for relationships between ejaculate quality and age, tenure, and social rank, we used similar linear models, after square-root transforming social rank and tenure. To compare ejaculate quality among the different age classes of immigrant males, we performed a multivariate analysis of variance (MANOVA). To compare immigrants with natal males, we first used 2-tailed non-parametric Mann-Whitney U-tests on each trait; we then tested for overall differences between immigrant males and natal males with a MANOVA.

We conducted all statistical analyses in R (R Development Core Team 2007). For the PCA and subsequent tests, we used the packages labdsv (Roberts 2006) and Lattice (Sarkar 2007). For calculating each ICC and its associated p-value, we used the package psych (Revelle 2010). Differences were considered significant when $p \leq 0.05$, and all means are reported with standard errors.

Results

Ejaculate quality in wild immigrant male spotted hyenas

We electroejaculated 16 immigrant males, all of which were older than 24 months and all of which produced a semen sample; no urine contamination was observed. Ejaculate trait means are reported in Table 2.1. The traits showing the most between-individual variation (CV) were sperm concentration (CV=128.8), total number of sperm (CV=123.9), percent motile sperm (CV=55.1), and total ejaculate volume (CV=40.92). The traits showing the least between-individual variation were sperm head length (CV=0.11), total sperm length (CV=2.9) and midpiece length (CV=6.2).

The PCA used to characterize overall ejaculate quality among the immigrant males yielded 2 components with eigenvalues ≥1 (Table 2.2), which was our criterion for inclusion in further analyses (Quinn & Keough 2002). In the first component, 3 input variables had eigenvectors greater than 70% of the largest eigenvector for that component, indicating that these traits contributed more heavily than did other variables (Mardia et al. 1979). The first principal component was driven by percent motile sperm, progressive status, and sperm concentration, and explained 58% of the total variation. Furthermore, in the first component, all of the traits loaded in the same direction. The second principal component was overwhelmingly driven by sperm length and explained 25% of the variation. Thus, together these 2 components explained 83% of the total variation in ejaculate quality.

Effect of testes size on ejaculate quality among immigrant males

We generated linear models to evaluate relationships between various aspects of ejaculate quality and testes size. There was no effect of testes size on either PC1 ($F_{1,14}$=1.598, p=0.23) or PC2 ($F_{1,14}$=0.078, p=0.78). Although there was a significant positive relationship between testes size and total ejaculate volume ($F_{1,12}$=15.220, p=0.002), there was no relationship between testes size and the total number of sperm ($F_{1,10}$=1.363, p=0.27).

Effects of social rank, tenure, and age on ejaculate quality among immigrant males

We used linear models to examine relationships between the first 2 principal component scores and social rank, tenure, or age of the immigrant males. There was no effect of social rank on PC1 ($F_{1,12}$=0.011, p=0.92) or PC2 ($F_{1,12}$=1.942, p=0.19), nor was there an effect of tenure on PC1 ($F_{1,6}$=0.501, p=0.51) or PC2 ($F_{1,6}$=2.376, p=0.17). We found no significant effect of age on PC1 ($F_{1,14}$=0.040, p=0.84), but PC2 showed a trend toward significance ($F_{1,14}$=3.839, p=0.07). Similarly, our MANOVA showed no significant effect of age class on ejaculate quality (Wilk's λ=0.465, $F_{8,22}$=0.834, p=0.58).

Effect of dispersal status on ejaculate quality

We electroejaculated 4 adult natal males, all of which were older than 24 months; every individual produced a semen sample, and no urine contamination was observed. Ejaculate trait means for the natal males are reported in Table 2.1. Due to the small number of adult natal males sampled, we used Mann-Whitney *U* tests to compare means in each trait between immigrant and natal males (Table 2.1). The mean values for immigrants were higher than those for adult natal males in every ejaculate trait, although not all differences were statistically significant. Concentration, percent motile, sperm length, ejaculate volume, total number of sperm, and testes size were all significantly greater in immigrants than in adult natal males (Figure 2.1). These general results were corroborated by the MANOVA on sperm concentration, percent motile, progressive status, total sperm length, and midpiece length: immigrant males had significantly higher ejaculate quality than adult natal males (Wilk's λ=0.579, $F_{5.14}$=3.845, p=0.02).

Most of the immigrant males here were older than the sampled adult natal males (Table 2.1), which precluded the possibility of an age-matched statistical comparison between immigrants and adult natal males. However, of the 16 immigrant males sampled, 2 had ages comparable to those of our adult natal male subjects. We could not statistically compare these 2 "young immigrant" males (mean age 30.5±6.5 months) to the group of natal males due to small sample sizes. However, we note that despite approximate age-matching, the mean values for the 2 young immigrants were higher than those for the 4 adult natal males in the following traits: concentration, percent motile, progressive status, sperm length, midpiece length, and sperm motility index.

Individual repeatability

To evaluate repeatability in ejaculate quality within individuals, we electroejaculated each of 6 captive hyenas twice. The mean age of the captive males was 154.33 (range 63-221) months, and mean values for the ejaculate traits of the captive hyenas are reported in the second row of Appendix B. The Spearman rank correlation coefficients demonstrated that sperm concentration, progressive status, and total ejaculate volume were all repeatable (Table 2.3; $R_S \geq 0.4$), but that percent motile sperm was not ($R_S=0.1$). The ICC values supported these findings in that they were >0.7 for sperm concentration (Table 3; $F_5=12.0$, $p=0.01$), progressive status ($F_5=5.8$, $p=0.04$), and total ejaculate volume ($F_5=9.0$, $p=0.03$), which indicates high repeatability (Measey et al. 2003); in contrast, the ICC value for percent motile sperm was approximately 0 ($F_5=0.9$, $p=0.57$).

Discussion

Here, we provide the first documentation of semen characteristics in wild and captive spotted hyenas. Aside from a single brown hyena (*Parahyaena brunnea*) electroejaculated once in 1981 (Ensley et al. 1982), this is the first time semen from any species in the family Hyaenidae has been collected and evaluated. Therefore, although our small sample sizes limit firm conclusions, this work nevertheless represents a useful contribution to the study of mammalian ejaculate quality.

Ejaculate quality in wild male spotted hyenas

Sperm concentration, percent motile, and progressive status all contributed substantially to the first principal component in our PCA of immigrant male ejaculates, which explained nearly half the variation in our data (Table 2.2). Furthermore, these traits, as well as sperm length, all loaded in the same direction. Previous research indicated that these traits correlate positively with fertilization success in other species (e.g., Birkhead et al. 1999; Powell et al. 2001; Denk 2005; Fitzpatrick et al. 2009), suggesting that PC1 here may serve as a proxy for overall ejaculate quality. PC2 was driven almost entirely by sperm length, which implies that sperm length varies independently from concentration, percent motile, or progressive status in this species. Future work should now test the fundamental assumption of sperm competition theory, which is that inter-male variation in ejaculate quality corresponds with differential fitness, as has been shown in other mammals (Holt et al. 1997; Gomendio et al. 1998; Ward 1998; Malo et al. 2005; Gomendio et al. 2006; Malo et al. 2006). We hope to do this for wild spotted hyenas by elucidating the relationship between ejaculate quality and paternity success in the litters potentially sired by the males we sampled.

Effect of testes size on ejaculate quality among immigrant males

Testes size is routinely used as an indicator of sperm activity, including total number of sperm and ejaculate volume (Gomendio et al. 1998; Preston et al. 2003; Schulte-Hostedde & Millar 2004). Here, however, we found no relationship between testes size and total number of sperm, although we did find a significant positive correlation between testes size and ejaculate volume. Additionally, testes size was not related to either principal component, implying that there may be no relationship between testes size and overall ejaculate quality in this species.

What remains unclear is whether this lack of correlation between testes size and ejaculate quality is related to the fact that spotted hyenas have unusually small testes relative to their body size (Iossa et al. 2008; Soulsbury 2010). This may be due to phylogeny, given that members of the family Hyaenidae have the smallest mean RTS among carnivores, and most carnivore species have smaller RTS than do species in other mammalian orders (Kenagy & Trombulak 1986; Iossa et al. 2008). Furthermore, *Crocuta crocuta* first appear in the fossil record only 990,000–250,000 years ago, and descended from solitary ancestors (Werdelin & Lewis 2000); thus, it is possible that sperm competition was not historically a selective force in this genus, and that the testes morphology of extant spotted hyenas is undergoing an evolutionary lag. Alternatively, Soulsbury (2010) speculated that female control over copulation in this species might reduce the intensity and importance of post-copulatory competition, thereby reducing selection on testes size, and our data showing no correlation between ejaculate quality and testes size support this possibility. Nevertheless, the well-documented promiscuity among female hyenas (Szykman et al. 2007) and the frequency of multiple paternity in hyena litters (Engh et al. 2002; East et al. 2003) both suggest that sperm competition may be important in this species, indicating that testes size may not always be as reliable an indicator of sperm competition as is commonly believed (Reynolds et al. 2004; Firman & Simmons 2010). Future work exploring a relationship between ejaculate quality and paternity success should help resolve this apparent contradiction.

Effects of social rank, tenure, and age on ejaculate quality among immigrant males

Previous research has shown that, although social rank is correlated with male reproductive success in spotted hyenas, the highest-ranking males have lower reproductive success, and lower-ranking males have higher reproductive success, than would be predicted by a rank-based priority of access model (Engh et al. 2002). One possible explanation for this is that socially subordinate males compensate for their reduced access to copulations by investing more heavily in their ejaculates than do their dominant counterparts, thus improving their paternity success via sperm competition (Parker 1990), as has been demonstrated in other species (Stockley & Purvis 1993; Vladic & Jarvi 2001; Schulte-Hostedde & Millar 2004; Cornwallis & Birkhead 2006; Serrano et al. 2006). However, our data fail to support this hypothesis, because neither of the first 2 principal components in our analysis, which together accounted for 83% of the variation in ejaculate quality, was correlated with social rank.

Reproductive senescence has also been suggested to explain the surprisingly low paternity success among the highest-ranking male hyenas, because these males are often quite old (East et al. 2003). Many male mammals show declines in reproductive fitness as they reach old age (Wang et al. 1993; Wolf et al. 2000; Kidd et al. 2001; Thongtip et al. 2008); however, we found no significant differences in ejaculate quality among the 3 immigrant age classes (young, middle-age, and old). Our results therefore fail to support the hypothesis that reproductive senescence explains low paternity success among the highest-ranking males, but are consistent with data from the few other studies on ejaculates of wild carnivores, which also showed no decline in ejaculate quality among older adult males (Brown et al. 1991; Crosier et al. 2007; Gañán et al. 2010). Given that most examples of reproductive senescence come from studies on captive animals, senescence may be an artifact of the unnaturally long lives of captive animals.

Effect of dispersal status on ejaculate quality

Our results support the hypothesis that dispersal status affects ejaculate quality: we found viable sperm in the ejaculates of all adult natal males sampled, so they theoretically could have sired cubs, but their overall ejaculate quality was substantially lower than that of immigrants, including the two immigrants who were of comparable age to the adult natal males. In fact, immigrant males were superior to adult natal males in every ejaculate trait we measured. These findings are consistent with the hypothesis that adult natal males may be experiencing physiological reproductive suppression, which was originally proposed by Holekamp & Sisk (2003) when they found that immigrant male spotted hyenas have significantly higher testosterone levels than age-matched natal males. They also suggested that the difference in testosterone concentrations between adult natal and immigrant males might be associated with differences in their ejaculate quality, because a positive relationship between testosterone concentrations and ejaculate quality has been well established in other mammals (Johnston et al. 1994; Meeker et al. 2006; Kishk 2008; Minter & DeLiberto 2008).

Individual repeatability

We were only able to sample a small number of captive male hyenas twice, so our conclusions regarding repeatability must be viewed with caution. Nevertheless, in our analysis of the repeatability of ejaculate quality within individual males, the use of 2 different statistical techniques consistently demonstrated that concentration, progressive status, and total ejaculate volume were all repeatable (Table 2.3). Our findings are thus consistent with previous research demonstrating individual consistency in ejaculates in other species (Morrow & Gage 2001; Birkhead et al. 2005). Evolutionary inferences about ejaculate quality are based on the assumption that variation among individuals is consistent over time, but this assumption is rarely tested. By showing here that individual ejaculate quality was indeed consistent over time, we can deduce that the variation observed here among wild hyenas can likely be attributed to true individual differences. This conclusion should be encouraging to researchers studying species that cannot easily be sampled more than once in the wild, such as large carnivores.

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Figure 2.1. Traits in which we found significant differences between wild adult natal and immigrant male spotted hyenas (*Crocuta crocuta*). Groups were compared using Mann-Whitney *U* tests ($\alpha = 0.05$). Horizontal lines indicate medians, box edges show 1st and 3rd IQR, and whiskers represent closest data points within (1.5 x IQR) of box edges. A = Sperm concentration; B = Percent motile; C = Sperm length; D = Total ejaculate volume; E = Total number of sperm; F= Testes size (length × width). In (A), (E), and (F), a single outlier point in the immigrant male group is not shown but was included in all statistical analyses.

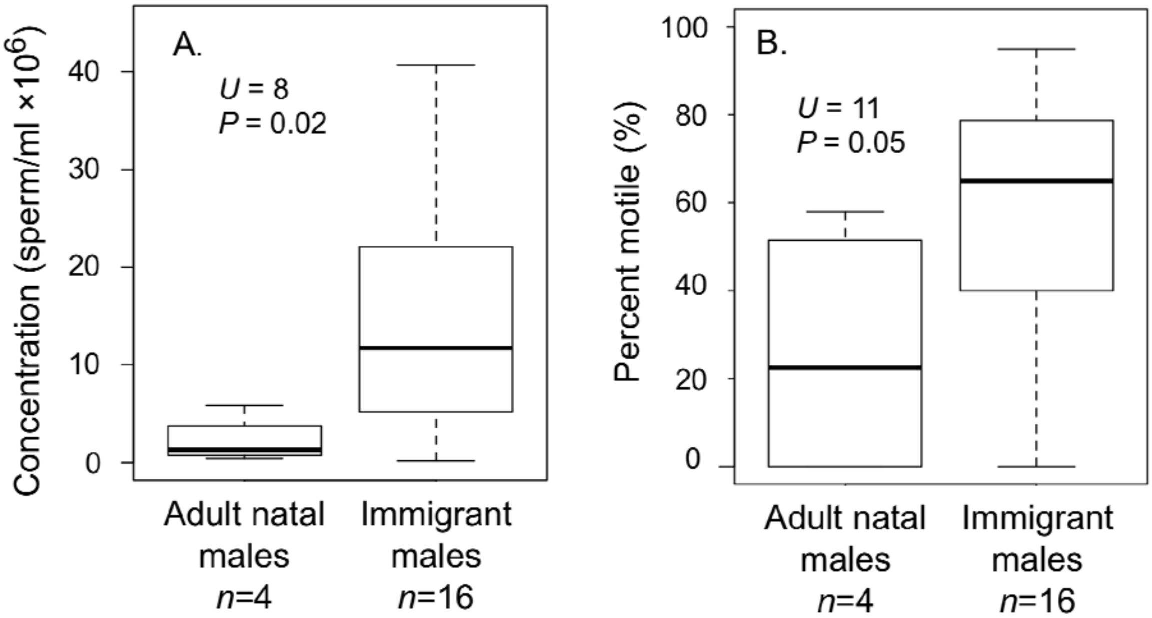

Figure 2.1 cont.

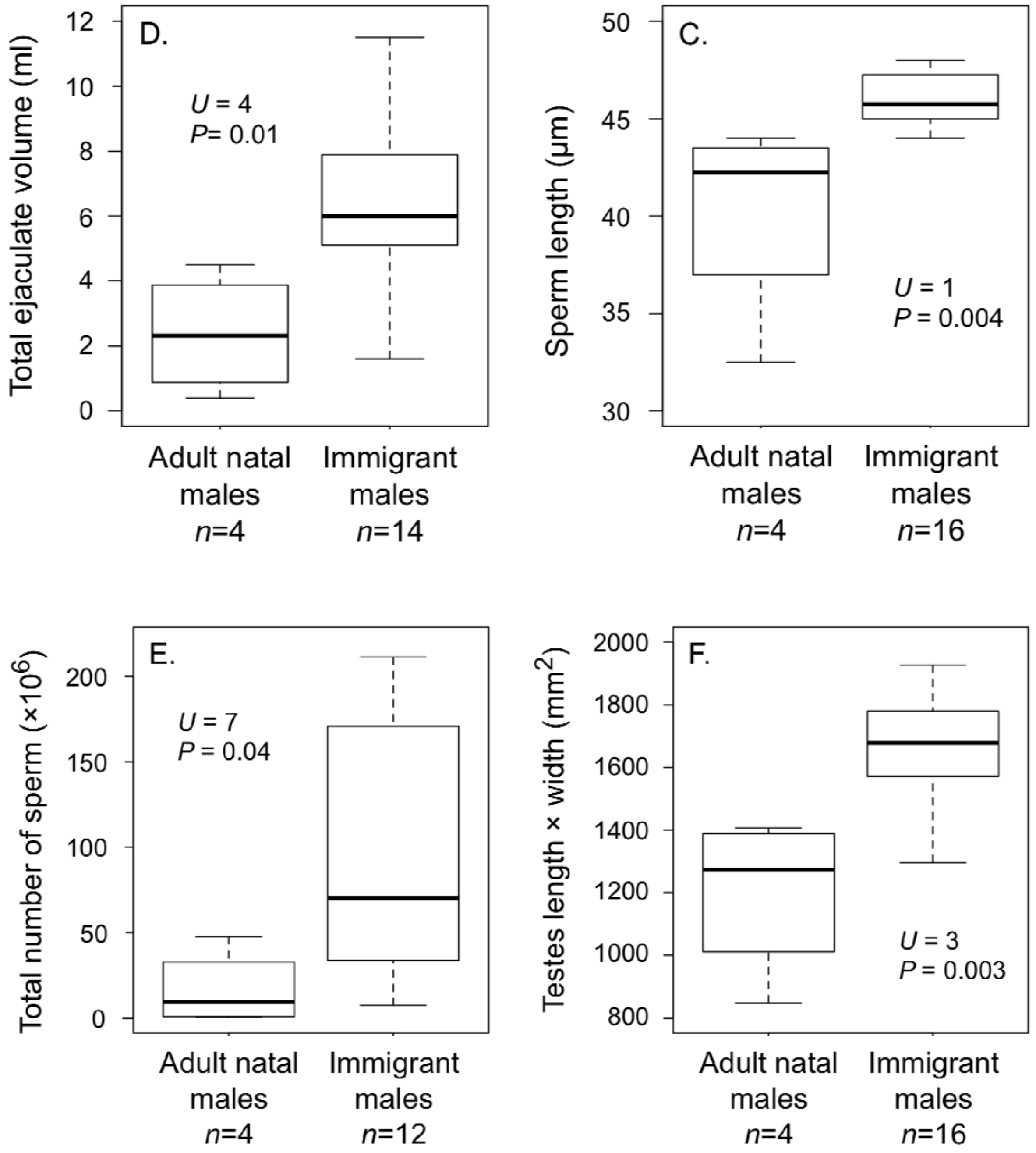

Table 2.1. Mean (± s.e.m.) ejaculate characteristics of wild immigrant and adult natal male spotted hyenas (*Crocuta crocuta*) and comparisons between the two groups. We used Mann-Whitney *U* tests to compare means between immigrants and adult natal males. Ages are reported with standard errors and parenthetical ranges. Additionally, because progressive status is an ordinal variable, for this trait we present the medians (and parenthetical first and third quartiles) instead of the means.

Ejaculate trait	Immigrant males (n=16)	Adult natal males (n=4)	M-W *U*	*p*-value
Age (months)	89.8 ± 10.61 (24-191)	28.5 ± 1.85 (24-33)	-	-
Concentration (sperm/mL x10^{6})	18.52 ± 5.96	2.23 ± 1.24	8	**0.02**
Percent motile	56.47 ± 7.78	25.75 ± 15.10	11	**0.05**
Progressive status[a]	4.0 (3.5, 5)	2.0 (0, 4)	15	0.11
Sperm length (µm)	45.97 ± 0.33	40.25 ± 2.63	1	**<0.01**
Sperm midpiece length (µm)	5.81 ± 0.09	5.50 ± 0.20	17	0.11
Sperm head length (µm)	3.75 ± 0.10	3.50 ± 0.29	24	0.40
Sperm motility index[b]	66.73 ± 6.52	33.50 ± 19.36	13	0.08
Total ejaculate volume (mL)	6.40 ± 0.76	2.38 ± 0.92	4	0.01
Total number of sperm x10^{6}	123.57 ± 44.18	16.74 ± 11.07	7	0.04
Testes length x width (mm^{2})	1685.58 ± 55.27	1200.30 ± 127.89	3	<**0.01**

[a] Rate of movement on a scale of 0 (no movement) to 5 (rapid, forward progress).

[b] SMI = (percent motile + [20 x progressive status])/2.

Table 2.2. Principal component analysis of sperm traits in immigrant male spotted hyenas (n=16).

Trait	PC1[a]	PC2
Concentration	**-0.53**[b]	0.25
Percent motile	**-0.60**	-0.04
Progressive status	**-0.57**	0.08
Sperm length	-0.17	**-0.96**
Variance explained (%)	0.58	0.25
Total variance explained (%)		0.83

[a] Only components with eigenvalues >1 are shown.

[b] Eigenvectors in bold are >70% of the largest eigenvector, and therefore contribute heavily to that component (Mardia et al. 1979).

Table 2.3. Indices of repeatability in semen traits of captive male spotted hyenas.

Trait[a]	Spearman rank (R_s)	ICC[c]	ICC *p*-value
Concentration	0.89	0.85	<0.01*
Percent motile	0.12	-0.08	0.57
Progressive status[b]	0.66	0.71	0.04*
Total ejaculate volume (ml)	0.41	0.80	0.03*

[a] n=6 for all traits except total ejaculate volume (n=5).

[b] Rate of movement on a scale of 0 (no movement) to 5 (rapid, forward progress).

[c] Intraclass correlation coefficients. *Statistically significant at α=0.05.

APPENDIX

APPENDIX B

Table B1. Mean ejaculate traits (± s.e.m.) of 43 carnivore species.

Species	RTS[a]	W/C[b]	Conc. (sperm/mL x10^6)	% Motile	Prog. status[c]	% Normal morph.	Sperm length (µm)	References
Crocuta crocuta, spotted hyena	0.0219	W	18.5±6.0	56.5±7.8	3.8±0.3	-	46.0	current study, RTS from Iossa et al. 2008
Crocuta crocuta, spotted hyena	0.0219	C	10.8±4.7	43.7±6.9	2.9±0.6	-	-	current study, RTS from Iossa *et al*. 2008
Parahyena brunnea, brown hyena	-	C	270.0	80.0	4.0	-	-	Ensley *et al*. 1982, Soulsbury & Iossa 2010
Panthera tigris, Siberian tiger	0.0072	C	20.3±2.8	60.4±2.5	-	78.3±1.7	51.4	Byers *et al*. 1990, Schmehl *et al*. 1990, Anderson *et al*. 2004, Anderson *et al*. 2005, Soulsbury & Iossa 2010
Panthera pardus fusca, Indian leopard	-	C	55.8±11.7	57.1±5.1	-	71.9±4.6	62.1	Gage 1998, Jayaprakash *et al*. 2001, Soulsbury & Iossa 2010
Panthera pardus kotiya, Sri Lankan leopard	-	C	13.1±5.5	55±5.2	3.3±0.2	22.8±5.8	-	Brown *et al*. 1989
Panthera leo, lion	0.0293	W	12.3±3.8	89±2.1	4.1±0.3	71.5±4.8	-	Brown *et* al. 1991, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Panthera onca, jaguar	0.0350	W	35±21.3	73±6.1	3.5±0.2	73.5±3.9	-	Morato *et al*. 2001, Iossa *et al*. 2008

Table B1 (cont'd)

Panthera onca, jaguar	0.0350	C	4.3±1.7	64±2.4	2.8±0.1	57.3±5.0	-	Morato *et al*. 2001, Swanson *et al*. 2003, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Panthera uncia, snow leopard	0.0190	C	38.7±5.3	76.3±2.1	3.8±0.1	56.7±2.8	-	Roth *et al*. 1996, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Neofelis nebulosa, clouded leopard	0.1208	C	27.5±2.3	71±2.1	3.9±0.1	61.1±1.7	-	Wildt *et al*. 1986, Anderson *et* al. 2004, Soulsbury & Iossa 2010
Leopardus pardalis, ocelot	0.1985	C	53.8±17.9	81.4±1.2	3.7±0.1	58.4±5.8	-	Morais *et al*. 2002, Swanson *et al*. 2003, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Leopardus tigrinus tigrinus, oncilla	0.1099	C	83.0±35.5	71.4±2.3	3.8±0.1	35.6±6.0	56.3	Morais *et al*. 2002, Swanson *et al*. 2003, Anderson *et al*. 2005, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Leopardus wiedii, margay	0.1466	C	14.2±5.3	73.5±1.3	3.4±0.1	39.5±7.7	-	Morais *et al*. 2002, Swanson *et al*. 2003, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Leopardus colocolo, pampas cat	0.0640	C	364.0±326.0	-	-	56.5±0.5	-	Swanson *et al*. 2003, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Lynx rufus, bobcat	0.0304	C	24.4±7.8	55.7±5.8	2.7±0.2	14.7±2.1	54.5	Anderson *et al*. 2005, Iossa *et al*. 2008, Gañan *et al*. 2009, Soulsbury & Iossa 2010
Lynx pardinus, Iberian lynx	0.1756	W	10.1±4.1	58.3±6.3	2.7±0.3	25.9±6.0	-	Gañan *et al*. 2010
Lynx pardinus, Iberian lynx	0.1756	C	20.5±6.0	85.6±2.3	3.3±0.1	33.0±4.3	-	Gañan *et al*. 2010
Lynx lynx, Eurasian lynx	0.0367	C	7.6±3.6	57.5±21.4	-	25.8±10.3	-	Jewgenow *et al*. 2006, Iossa *et al*. 2008, Soulsbury & Iossa 2010

Table B1 (cont'd)

Puma concolor, puma	0.0331	C	15.6±4.5	-	-	33.5±3.4	-	Swanson *et al*. 2003, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Herpailurus yaguaroundi, jaguarundi	0.0391	C	7.2±4.0	-	-	25.7±4.6	-	Swanson *et al*. 2003, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Acinonyx jubatus, cheetah	0.0189	W	23.8±5.2	68±3.4	3.4±0.1	21.1±2.6	-	Anderson *et al*. 2005, Crosier *et al*. 2007, Iossa *et al*. 2008
Acinonyx jubatus, cheetah	0.0189	C	29.3±5.6	67±2.0	3.6±0.1	21.3±2.0	56.0	Gage 1998, Crosier *et al*. 2007, Iossa *et al*. 2008
Prionailurus viverrinus, fishing cat	0.0363	C	108±29.0	73±4.0	4.0±0.2	33.5±6.8	-	Thiangtum *et al*. 2006, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Prionailurus bengalensis, leopard cat	-	C	37.0±5.4	73.8±2.6	3.5±0.1	65.4±2.0	-	Howard & Wildt 1990, Soulsbury & Iossa 2010
Otocolobus manul, Pallas' cat	0.0275	C	123.0±16.7	-	-	63.4±2.0	-	Swanson *et al*. 1996, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Felis nigripes, black-footed cat	0.0725	C	130.4±23.6	82.5±1.9	3.6±0.1	46.7±3.0	50.5	Anderson *et al*. 2005, Iossa *et al*. 2008, Herrick *et al*. 2010
Felis catus, domestic cat	0.0757	C	223.8±73.3	66.9±10.8	4.1±0.6	70.9±3.7	59.5	Wildt *et al*. 1983, Griffin 2001, Iossa *et al*. 2008, Zambelli *et al*. 2010
Felis margarita, sand cat	0.0366	C	209.8±38.3	78.3±1.3	3.4±0.1	40.4±3.1	-	Crissey *et al*. 1997, Herrick *et al*. 2010, Soulsbury & Iossa 2010
Lycaon pictus, African wild dog	0.0986	C	212.3±87.8	69.5±3.3	3.4±0.3	76.2±6.2	60.0	Anderson *et al*. 2005, Johnston *et al*. 2007, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Canis latrans, coyote	0.1453	C	549.2±297.7	90.4±4.5	-	78±13.5	55.1	Anderson *et al*. 2005, Iossa *et al*. 2008, Minter & DeLiberto 2008

Table B1 (cont'd)

Canis rufus, red wolf	0.1302	C	146.5±25.7	71.2±6.0	-	73.6±3.2	-	Goodrowe *et al*. 1998, Kalinowski *et al*. 1999, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Canis lupus lupus, gray wolf	0.0835	C	271.7±59.4	98.8±0.4	-	77.0±3.4	61.6	Gage 1998, Zindl *et al*. 2006, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Canis lupus familiaris, domestic dog	0.1260	C	129.6±6.9	30.1±0.9	-	-	60.7	Ohl *et al*. 1994, Gage 1998, Anderson *et al*. 2005, Iossa *et al*. 2008
Ailuropoda melanoleuca, giant panda	0.2392	C	-	80±4.3	2.8±0.2	72.5±5.1	51.2	Gage 1998, Olson *et al*. 2003, Anderson *et al*. 2005, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Ursus americanus, American black bear	0.0302	C	-	-	-	35.6±7.1	75.3	Anderson *et al*. 2005, Iossa *et al*. 2008, Brito *et al*. 2010
Ursus thibetanus, Asiatic black bear	0.0556	C	1049±936.8	63.8±26.3	3.5±0.5	62.1±28.5	-	Chen *et al*. 2007, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Ursus thibetanus japonicus, Japanese black bear	0.0513	C	221.4±49.5	77.1±4.2	-	50.3±12.4	-	Okano *et al*. 2006
Ursus arctos, brown bear	0.0509	C	519±71.8	73.9±3.7	-	62.0±4.1	73.8	Gage 1998, Anderson *et al*. 2005, Anel *et al*. 2008, Iossa *et al*. 2008
Ursus arctos yesoensis, Hokkaido brown bear	-	C	471.6±129.4	80.2±6.3	-	78.2±3.5	-	Ishikawa *et al*. 1998, Mano *et al*. 2002, Soulsbury & Iossa 2010

Table B1 (cont'd)

Spilogale gracilis, western spotted skunk	0.4707	C	132±16.0	56±2.4	-	70.3±1.5	-	Kaplan & Mead 1993, Verts *et al*. 2001, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Spilogale putorius, eastern spotted skunk	0.7479	C	-	68±4.0	-	64.3±1.7	-	Kaplan & Mead 1994, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Nasua nasua, S. American coati	0.0661	C	60.0	68.0	3.2	85.3	78.3	Iossa *et al*. 2008, Lima *et al*. 2009, Soulsbury & Iossa 2010
Lontra canadensis, N. American river otter	0.2903	C	273.6±147.1	90±0.0	3.6±0.3	71.2±13.3	-	Hamilton & Eadie 1964, Iossa *et al*. 2008, Bateman *et al*. 2009
Mustela eversmanni, steppe polecat	0.1039	C	18.1±2.9	93±6.0	-	27.0	73.7	Anderson *et al*. 2005, Iossa *et al*. 2008, van der Horst *et al*. 2009
Mustela nigripes, black-footed ferret	0.2022	C	1030.9±151.0	58.5±2.5	3.2±0.1	53.2±3.1	-	Gage 1998, Wolf *et al*. 2000, Iossa *et al*. 2008, Soulsbury & Iossa 2010
Mustela putorius furo, domestic ferret	0.3323	C	706.1±50.5	80.7±1.0	3.3±0.1	67.3± .3	-	Howard *et al*. 1991, Iossa *et al*. 2008, Soulsbury & Iossa 2010

[a] Relative testes size (RTS) = testes mass/total body mass)*100.

[b] Wild (W) or captive (C) study.

Table B1 (cont'd)

[c] Rate of movement on a scale of 0 (no movement) to 5 (rapid, forward progress). Although progressive status is an ordinal variable, making any calculations of means statistically erroneous, we have reported means here for the sake of comparison with other species for which means and standard errors of the mean have been reported. For wild *Crocuta crocuta*, the median of progressive status was 4.0, with the middle 50% of samples falling between 3.5 and 5.0.

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CHAPTER 3
THE RELATIONSHIP BETWEEN TENURE AND REPRODUCTIVE SUCCESS AMONG MALE SPOTTED HYENAS

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Abstract

In most cases of endurance rivalry, males compete to remain reproductively active longer than other males, but these time periods are typically brief, such as a single breeding season. Here, we explored endurance rivalry among immigrant males in a species that breeds year-round, the spotted hyena (*Crocuta crocuta*). We found that most males were present in the clan for over two years before siring their first cub, and that the majority of males either sired their first cub in the first four years of their tenure, or did not sire one at all. Next, we examined how long immigrants remained in the clan before disappearing, and found that most immigrants that sired at least one cub remained in the clan at least four years, whereas males that never sired any cubs typically disappeared by the fourth year of their tenure. This suggests that males might incorporate their initial reproductive success in the clan into their decision regarding whether to remain in the clan or secondarily disperse to another clan. Finally, we used Bayesian mixed modeling to explore the relationship between annual reproductive success and several potentially influential factors, including tenure. We found that annual male reproductive success increased during the first six years of a male's tenure, then decreased after six years, indicating that perhaps tenure is not the sole determining factor in a male's reproductive success. For example, we found a significant positive relationship between a male's annual reproductive success and his social associations with adult females, although we found no effect of his rate of aggressive interactions with these females. Our results support the notion that males compete intrasexually via an endurance rivalry in that we have shown unequivocally that tenure affects male reproductive success, but questions remain regarding other traits salient to the rivalry, which females might select for and against as they choose their mates.

Keywords: *Crocuta crocuta*, endurance rivalry, male reproductive success, MCMCglmm, spotted hyena, tenure

Introduction

When the females of a species have the limiting reproductive rate, males typically compete via one of four mechanisms of intrasexual competition: male-male combat, scramble competition, sperm competition, or endurance rivalry (Andersson et al. 1994). "Endurance rivalry" traditionally refers to a competition among males to remain reproductively active for as long as possible within a single breeding season and/or at a specific breeding site, with females demonstrating a mating preference for the winners of these "marathons" (Judge & Brooks 2001; Lidgard et al. 2005; Higham et al. 2011).

In the classic examples of this non-aggressive form of male-male competition, the outcome of the competition relies heavily on fat and energy reserves and physical stamina. For example, male bullfrogs (*Rana catesbeiana*) that call for a greater number of nights during the mating season obtain significantly more copulations than do males that call for fewer nights (Judge & Brooks 2001). The males that call the most are the individuals that entered the breeding season with the best body condition, but the exertion of the endurance rivalry reduces these superior males to the poorest body condition by the conclusion of the breeding season. Likewise, male gray seals (*Halichoerus grypus*) with higher body fat and higher body energy can sustain mating for longer throughout the mating season than can male seals with lower body fat, but virtually all males experience a significant decrease in percentage body fat between the beginning and end of the mating season (Lidgard et al. 2005). A similar pattern has been found in rhesus macaques, (*Macaca mulatta*): males establish their body condition during the non-breeding season, and high-ranking males enter the breeding season in the best condition (Higham et al. 2011). These high-ranking males have the greatest mating success, but conclude the mating season in the poorest body condition.

At first, spotted hyenas (*Crocuta crocuta*) appear unlikely candidates for this type of endurance rivalry because they breed at all times of year (Kruuk 1972), so their marathon would appear to be virtually endless. Other factors, however, make an endurance rivalry seem quite plausible, especially if the concept of an endurance rivalry is expanded. Females have the limiting reproductive rate (Holekamp et al. 1996), and males show considerable reproductive skew (Engh et al. 2002), so male-male competition should theoretically be intense in this species (Darwin 1871). In spite of this prediction, male hyenas do not meet the criteria for scramble competition (Andersson et al. 1994) and exhibit relatively little male-male combat (East & Hofer 2001). Sperm competition remains a potential mechanism for intrasexual competition in this species (Curren et al. 2013), but male spotted hyenas may also compete via an endurance rivalry that hinges on more than the stamina required for a single breeding season (Judge & Brooks 2001; Lidgard et al. 2005; Higham et al. 2011).

After reaching puberty, male spotted hyenas ordinarily disperse from their natal clans and immigrate into new clans (Smale et al. 1997; Van Horn et al. 2003). Immigrants are subordinate to all natal individuals, and intrasexual social rank among immigrants is determined exclusively by tenure in their new clans (Holekamp & Smale 1998; East & Hofer 2001). New immigrants therefore enter a clan at the very bottom of the social hierarchy, such that their priority of access to food at kills is the lowest in the entire clan (Smale et al. 1997; Holekamp & Smale 1998; East & Hofer 2001). Previous research on spotted hyenas has demonstrated that tenure may be an important component of an immigrant male's reproductive success (Engh et al. 2002; East et al. 2003), but the magnitude and exact nature of this effect remain unclear. For example, East et al. (2003) found that males typically must wait two years in the clan before siring cubs, but the precise distribution of tenure at first paternity was not reported. It is also unclear how an immigrant’s reproductive success in the early years of his tenure might affect his decision to remain in the clan versus secondarily dispersing. Roughly 40% of immigrant male spotted hyenas appear to engage in secondary dispersal (Van Horn et al. 2003), and although the costs of this behavior appear to be enormous, the reasons for its occurrence are unknown. Our first objective was to fill these gaps in our understanding of the connection between male reproduction and dispersal.

Our second goal was to elucidate the relationship between tenure and annual reproductive success. If immigrant males do compete via an endurance rivalry, and tenure in the clan is the only salient feature of endurance, we would expect to see a positive linear relationship between tenure and annual reproductive success (hereafter annual RS). Engh et al. (2002) explored reproductive success as a function of tenure and reported a significant positive effect of tenure, but their data hinted at a more complex relationship (see their Figure 6). Likewise, East et al. (2003) reported that some males have a “period of genetic reproductive inactivity” at the end of their tenure during which they sire no cubs, but they did not address how tenure affects reproductive success any further than this.

Furthermore, there may be additional components of endurance aside from mere presence in the clan. We therefore explored other variables that might affect an immigrant male’s reproductive success. For example, perhaps endurance entails not only being present in the clan, but also spending time in close proximity to adult females (Bercovitch 1997; Szykman et al. 2001) in order to develop relationships with them, as suggested by East et al (2003). In this case, a male’s annual RS should increase as his associations with adult females increase (Bercovitch 1997), irrespective of his tenure. Finally, Szykman et al. (2003) suggested that aggressive interactions between males and females might affect the female hyena’s mate-choice decisions.

Therefore, we explored the possibility that endurance requires not only remaining in the clan for an extended period and associating closely with females, but also actively engaging in aggressive interactions with females (East et al. 2003). If this were the case, we would expect to see a significant relationship between a male's annual RS and the rate at which he directs aggression toward, or receives aggression from, adult females.

Methods

Subject population

The data analyzed here came from a single wild spotted hyena clan that was continually monitored from 1988-2009 in the Masai Mara National Reserve, Kenya. We used unique spot patterns and other markings to identify individuals (Frank 1986), and we determined the sex of each individual based on the morphology of its erect phallus (Frank et al. 1990). The birthdates of all natal animals were estimated (±7 days) using methods described previously (Holekamp et al. 1996). We considered a female to be an "adult" when she had reached 36 months of age or conceived her first litter, whichever occurred first (Smith et al. 2007).

Previous work has shown that the majority of adult males in a clan are immigrants born in other clans, and that these immigrants sire the vast majority (97%) of cubs in the clan (Engh et al. 2002). On rare occasions, however, a male remains in his natal clan throughout his adult life such that he never disperses, and he may then sire offspring in his natal clan (East & Hofer 2001). In our study population, of 99 natal males whose fates were known after puberty, only two failed to disperse. As has also been described in other study populations (East & Hofer 2001), these two adult natal males behaved like immigrant males except that they were the highest-ranking males in the immigrant queue. We observed these two adult natal males attempting to mate with females, and each successfully sired multiple cubs. Therefore, although we restricted our analyses to immigrant males, we included these two adult natal males in all our analyses and treated them as immigrant males; hereafter, “immigrant males” will include these two individuals.

We elected to include tenure, rather than social rank, in our analyses because the two parameters are highly correlated (Smale et al. 1997; East & Hofer 2001), but tenure contains additional information about an individual’s life history in the clan. Because we assessed reproductive success on an annual basis using calendar years, we assigned immigrants a “rounded tenure” for each year in which they resided in the clan. If an immigrant’s arrival date in the clan was between January 1 and June 30, we assigned him tenure=1 for that year, and the next calendar year was tenure=2, etc. If his arrival date in the clan was between July 1 and December 31, we did not assign him a tenure for that year and his first official year of tenure (tenure=1) was the following calendar year. Tenure=1 therefore reflects approximately the first twelve months of an immigrant’s tenure; tenure=2 represents months 12-24, etc. For the two adult natal males that never dispersed, we began their tenure as “immigrants” on their second birthday, because male spotted hyenas achieve reproductive maturity late in their second year (Glickman et al. 1992). Each male was assigned a tenure for his final calendar year in the clan regardless of the month during that year in which he disappeared. We excluded from our analyses all immigrants that arrived in the clan before the inception of the study in 1988, males that remained in the clan for less than six months, and males for which we lacked genetic data.

Behavioral data collection

All behavioral observations were conducted from vehicles for several hours each morning and evening. We initiated an observation session when we encountered a subgroup of one or more hyenas that were separated from others by at least 200m (Smith et al. 2008). We first recorded the identity and activity of all hyena(s) present, and then used all-occurrence sampling methods to record certain behaviors as critical incidents (Altmann 1974). These critical incidents included the following behaviors, which were all classified as "aggressive acts": bites, bite-shakes, chases, lunges, pushes, stand-overs, "points" (aggressive posturing), head waves, and displacements. We also recorded the identity and sex of the target of the aggression. We considered an aggressive act to be dyadic if there was only one aggressor, and coalitionary if there were multiple aggressors cooperating to attack the same target.

To calculate annual rates of agonistic interaction between males and females, we first divided intersexual interactions into three categories: immigrant males directing dyadic aggression against adult females, immigrant males directing coalitionary aggression against adult females, and adult females direction aggression (dyadic or coalitionary) against immigrant males. We elected to separate dyadic aggression by males against females from coalitionary aggression by males against females because previous research has suggested that female spotted hyenas might respond differently to these two types of aggression from males (Szykman et al. 2003). Then, for each session in which an immigrant male was present with at least one adult female, we counted how many times he directed dyadic aggressive acts toward females, and how many times he was the target of aggressive acts by females. During each observation session in which an immigrant male was present with at least one adult female and at least one other immigrant male, we counted how many times he exhibited coalitionary aggressive acts against females. We then divided each count by the number of females present in the session to control for the number of opportunities to aggress or to receive aggression. Finally, we divided these numbers by the length of the session (limiting the analyses to sessions ≥15 minutes), thereby arriving at a rate of each type of aggression in each session for each immigrant present. We then averaged each of these three rates for each male across all sessions during each calendar year. In these calculations, we only included years in which a male was seen with an adult female (and, in the case of the rate of coalitionary aggression, with an adult female and at least one other immigrant male) at least ten times.

Genetic analysis of paternity

We collected DNA samples by anesthetizing individuals with Telazol (W.A. Butler Co., Brighton, MI, USA, 6.5 mg/kg) and extracting blood samples. We extracted DNA from blood using Puregene kits (Gentra Systems Inc., Minneapolis, MN, USA) and stored it in liquid nitrogen until it could be fully processed in the U.S. (Engh et al. 2002). We amplified and analyzed eleven autosomal microsatellite loci (CCr01, CCr04, CCr05, CCr07, CCr11, CCr12, CCr13, CCr14, CCr15, CCr16, and CCr17; GenBank Accession nos. AY394080-AY394084 and AF180491-AF180497) and one X-linked microsatellite locus (CCrA3; GenBank Accession nos. AY394085-AY394086) using conditions described previously (Libants et al. 2000; Engh et al. 2002).

To evaluate paternity, we first estimated the conception date of each natal animal by subtracting 110 days (the known gestation period; Holekamp et al. 1996) from each cub’s birthdate. We calculated a conception period for each litter ranging from six months before, to two months after, the date of conception. This broad range was a conservative estimate that allowed for uncertainty in a male’s presence/absence in the clan, and for errors in estimating conception dates. We considered as potential fathers any immigrant, transient (present in the clan less than 6 months), or adult natal male unrelated to the litter’s mother that were present in the clan during the conception period of a specific litter. Natal males were classified as unrelated to the mother when their coefficient of relatedness was <0.125 based on known maternal genealogies; natal males were classified as adults if they were at least 36 months old by the end of the conception period. We then assigned paternity to offspring using the program CERVUS (Marshall et al. 1998) with these input parameters: number of candidate fathers=25 (90% of candidate fathers sampled, because we lacked genetic information for some candidate fathers), 88% of loci typed, and an input error rate of 1%. We only included individuals that had been genotyped at a minimum of six loci, and only considered a male to be the father of an offspring when he was assigned paternity by CERVUS with 95% confidence.

Analyses of tenure at first paternity and tenure at disappearance

We first created frequency distributions of males' tenure when they first sired cubs and numbers of immigrants that disappeared during each tenure year without ever having sired any cubs. Then, to explore the relationship between reproductive success and immigrants' tenure in the clan, we created density plots to compare the distributions of males that sired at least one cub and males that never sired any cubs at all. In this analysis, we excluded all males that had not yet disappeared at the conclusion of our study (2009).

Exploring possible predictors of reproductive success with a statistical model

Here we modeled a male's annual RS, measured as the number of cubs to which he was assigned paternity in any given year. We created a Bayesian Markov Chain Monte Carlo generalized linear mixed model, assuming a Poisson distribution, with the R package MCMCglmm (Hadfield 2010). Because hyenas must become independent of the den to be darted, we only had genetic data for offspring that had achieved den independence, so this measure of reproductive success did not include cubs that died prior to den independence. To account for variation in the repeated measures we had for each male (one measure for each year during which he resided in the clan), we included a hyena's individual identity (ID) as a random effect in our model.

The fixed effects in our model reflected the variables that we identified as possible predictors of male reproductive success. First, we included a male's rounded tenure for the year in question, as well as a quadratic effect of tenure (tenure^2). Next, to address the possibility that social proximity to females is an additional component of endurance, we included the mean number of adult females present in sessions in which the male was observed. Then, to explore aggressive interactions as possible correlates of male reproductive success, we included the following fixed effects: the mean rate of dyadic aggressive acts directed toward adult females per session during a given year, the mean rate of coalitionary aggressive acts directed toward adult females per session during a given year, and the mean rate of aggressive acts (dyadic or coalitionary) received from females per session during a given year. Finally, we included how many total cubs were assigned paternity in a given year as a fixed effect to account for the variation in the number of possible cubs a male might theoretically have sired during any given year. Because we could not assign paternity to any cubs sired in 1989, we excluded this year from the model. We standardized all continuous covariates (tenure, tenure^2, all three rates of agonistic interactions, and number of cubs assigned) to have zero means and unit variances before their inclusion in the model.

For all fixed effects, we report the posterior mean estimates of the coefficients for each parameter, the 95% credible intervals (CI), and the *p*MCMC values. We considered a fixed effect to be statistically significant when its 95% CI did not overlap with zero. We report the proportion of variance explained by the random effect relative to the total variance in the model, expressed as a percentage. We report all descriptive means with the standard error of the mean and the range.

We ran the model for 500,000 iterations with a burn-in period of 200,000 and a thinning interval of 300, resulting in 1000 posterior samples comprising the posterior distribution of the model; these samples were used to generate all posterior estimates. We verified that the model had very low autocorrelation (<0.07 for successive iterations) for both the fixed effects and the variance component (Plummer et al. 2006; Hadfield 2010), and that it reached proper convergence, which we assessed both visually (using trace plots) and computationally (using Geweke's convergence diagnostic; Plummer et al. 2006). To assess convergence among multiple chains, we used potential scale reduction factors on three parallel (i.e., independent but identical) MCMC chains (Gelman & Rubin 1992). The posterior distribution and estimates were taken from the first of these chains. To confirm that there was no multicollinearity among our fixed effects, we calculated variance inflation factors and condition indices, verifying that variance inflation factors were all <2 and that the condition index was <5 (Heiberger 2009; Hendrickx 2010). Finally, the prior we used relied on the default prior for the fixed effects, which was a normal prior centered around zero and with a large variance (Hadfield 2010). For the random effect and the residual variance, the prior had a variance equal to half of the variance of the response variable, with a degree of belief (nu) equal to one. We tried multiple alternative priors, but the trace plots of the variance components indicated that they did not fit the data as well as the normal prior we used. We used R v. 2.13.0 to conduct all statistical analyses (R Development Core Team 2011).

Results

There were 53 immigrant males for which we had genetic data that entered the clan after the start of our study period (1988) and remained in the clan at least 6 months. Of these 53 immigrants, 43 disappeared before the conclusion of our study (2009). The mean length of tenure in the clan among these 43 immigrants was 4.37±0.37 years (range: 0.67-9.35), although the 10 immigrants that were still present in the clan at the conclusion of our study included two males that had been in the clan for 10 and 15 years, respectively.

Of the 250 offspring included in the paternity analysis, we were able to assign paternity at 95% confidence to 176 (70.4%), 172 (97.7%) of which had known, genotyped mothers. The mean observed error rate across all loci was 2.5%. We assigned paternity to 8.24±1.07 cubs per year (range: 1-19), and each immigrant sired an average of 0.60±0.07 cubs per year (range: 0-5). Among the 53 immigrants, 34 sired at least one cub during their tenure in the clan, and 19 never sired any cubs. On average, immigrants were present in the clan for 2.41±0.29 years (range: 0-8.20) before siring their first cubs (Figure 3.1). It is also apparent that a number of immigrants disappeared during each tenure year without ever having sired any cubs (Figure 3.1).

Among the 43 males for which we had disappearance dates, the total number of cubs sired during a male's lifetime tenure in the clan was 2.93±0.59 cubs (range: 0-18). The 26 immigrants that sired at least one cub during their tenure in the clan disappeared after a mean of 5.60±0.40 years (range: 2.48-9.35), whereas the 17 immigrants that never sired any cubs disappeared after a mean of 2.48±0.40 years (range: 0.67-7.41; Figure 3.2).

The model examined the annual RS of 48 individual immigrants over the course of their lifetime in the clan (limiting our analyses to years in which a male was seen with an adult female at least ten times resulted in the loss of 5 males). The complete model results are reported in

Table 3.1. Because the model used a log-link function, the reported coefficients are log-transformed. One must therefore take the inverse of the natural log of the reported value in order to interpret the absolute values of the coefficients. For example, the intercept indicates that a male that had the mean tenure, associated with the mean number of females per session, directed the mean rate of dyadic aggressive acts toward females, directed the mean rate of coalitionary aggressive acts toward females, received the mean rate of all aggressive acts from females, in a year in which the mean number of cubs were assigned paternity, would be expected to sire 0.53 cubs per year. One could perform similar back transformations for other covariates in the model.

There was a significant negative quadratic effect of tenure on annual RS (Figure 3.3), with a peak annual RS at tenure = 5.95 years. Although our sample sizes for tenure>9 were miniscule (n=2), limiting the conclusions we can draw about the annual RS of very high tenures, annual RS steadily declined between years 6-9, for which we had more robust sample sizes (tenure=6: n=19; tenure=7: n=15; tenure=8: n=9; tenure=9: n=6). We also found a significant positive relationship between the mean number of females present in each session with a given male and his annual RS. There was no significant effect of any of the three types of aggressive interactions (dyadic aggression directed by males toward females, coalitionary aggression directed by males toward females, or all aggression directed by females toward males) on annual RS. As expected, there was a significant positive correlation between the total number of cubs to which we could assign paternity each year and annual RS. Finally, hyena identity accounted for 25.348% of the total variance.

Discussion

Here we showed that an immigrant male spotted hyena must remain in a clan for an average of 2.4 years before siring his first cub, so time present in the clan clearly represents an important part of the endurance contest among male spotted hyenas. Most immigrants that sire cubs in a clan do so for the first time by their fourth year of tenure (Figure 3.1), and the majority of these males remain in the clan for 4-8 years (Figure 3.2). In contrast, immigrants that never sire any cubs in the clan typically disappear 1-4 years after they arrive (Figure 3.2).

The juxtaposition of Figures 3.1 and 3.2 may depict a reproductive strategy previously undetected in spotted hyenas. Most males that eventually sire at least one cub in the clan do so within the first four years (Figure 3.1), and most males that never sire any cubs disappear by the fourth year (Figure 3.2, dashed line). Together, these results suggest that perhaps a single factor is causing both a male's failure to sire offspring and his subsequent early disappearance from the clan. However, this idea is contradicted by previous research showing that immigrants that remained in the clan for several years were no more likely to have sired cubs during their first two years of tenure than were immigrants that disappeared during their third year of tenure (Engh et al. 2002). Engh et al. (2002) logically inferred from these data that males were unlikely to be electing to engage in secondary dispersal purely because they failed to sire cubs in their first two years in the clan. However, the sample size in their analysis (n=14 males) was considerably smaller than ours (n=43), and they examined siring success only during the first two years of tenure, rather than the first four. Perhaps, then, males are indeed making decisions regarding secondary dispersal based on their early siring success in the clan, and Engh et al.'s definition of "early" as meaning the first two years was simply too narrow. The data we have presented here are consistent with this hypothesis.

This notion is particularly intriguing given that most males do not emigrate from their natal clan until they are between 2-5 years old (Smale et al. 1997; Van Horn et al. 2003; Boydston et al. 2005), and males rarely live longer than 15 years in the wild (Drea & Frank

2003). This means that immigrants could be devoting as much as one third (or, in some cases, close to half) of their post-pubertal lifespan to a reproductive effort that is ultimately fruitless before deciding to renew their effort in a different clan by secondarily dispersing. However, theory predicts that the strategy of remaining in the clan to which they initially dispersed, despite not having early reproductive success, may still outperform the strategy of secondarily dispersing when the expected benefits of queuing are enhanced by the increased survival benefits of remaining in the clan (Kokko & Johnstone 1999). These additional survival benefits become proportionally more significant in long-lived species like the spotted hyena, and in species in which secondary dispersal imposes great mortality risk, as it does in the spotted hyena (Smale et al. 1997; Kokko & Johnstone 1999). In spite of this, our data indicate that few males sire their first cub after four years of tenure (Figure 3.1); at that point, secondary dispersal might be a better strategy.

We found clear support for the idea that male spotted hyenas compete via an endurance rivalry. In addition to surviving the high mortality risks associated with dispersing (Smale et al. 1997), a male must then endure the restricted food access that accompanies his new position at the bottom of a hierarchy containing as many as 125 other individuals (Kruuk 1972). As demonstrated here (Figure 3.1) and in East et al. (2003), a male must survive these conditions for an average of more than two years before he obtains any reproductive benefits at all. Furthermore, to attain peak reproductive benefits, he must survive and remain in the clan several more years (Figure 3.3). Taken together, these results plainly point to a contest among males to remain reproductively active for long periods of time, even with no initial reproductive benefits.

However, the quadratic relationship we found between annual RS and tenure (Figure 3.3) indicates that either females prefer males of intermediate tenures, in which case this "endurance rivalry" ends after approximately six years, or tenure is not the sole component of this endurance rivalry. This pattern, which is consistent with previous data on spotted hyenas (*see* Figure 6 in Engh et al. 2002) and with data from other mammals showing a similar relationship between age and annual RS (e.g., olive baboons, *Papio anubis*, Packer 1979; red deer, *Cervus elaphus*, Clutton-Brock et al. 1988; northern elephant seals, *Mirounga angustirostris*, LeBoeuf & Reiter 1988; Barbary macaques, *Macaca sylvanus*, Kuester et al. 1995; see Takahata et al. 1999 for review), is often ascribed to a decline in body condition with old age (Dunbar 1988), although Bercovitch et al. (2003) found no reduced body condition with age in rhesus macaques. Although the factors influencing body condition in spotted hyenas remain poorly understood, no evidence exists suggesting male body condition decreases after the sixth year of tenure. Furthermore, prior research has shown that ejaculate quality does not appear to decrease with age in spotted hyenas (Curren et al. 2013). Future research should therefore focus on determining the best indices of body condition in this species, and testing the hypothesis that males that remain in good body condition have higher reproductive success than those that remain in the clan for equal tenures but have poorer body condition (Bercovitch 1997; Higham et al. 2011).

The high degree of female control over copulation in this species (Cunha et al. 2003) suggests that female choice might be responsible for the observed decline in annual RS after the sixth year of tenure. Here, we did not directly test female preferences, but we did explore factors that could play a role in how females make their mating decisions. We found that males that were observed associating with a higher mean number of adult females had higher annual RS (Table 3.1), but we found no support, for the notion that a male's agonistic interactions with females influence his reproductive success. The rate at which adult females directed aggression toward a male did not affect his annual RS, nor did the rate at which the male directed dyadic aggression toward adult females. Most surprisingly, we found no relationship between the frequency with which a male directed coalitionary aggression toward adult females and his annual RS. Previous research pointed to a possible connection between aggressive coalitions of males targeting adult females and sexual selection, because females were the targets of these attacks more frequently when they were near the time of conception (Szykman et al. 2003). However, our results are not consistent with that hypothesis. Alternatively, perhaps affiliative, rather than aggressive, interactions between males and females affect male reproductive success (Szykman et al. 2001; East et al. 2003). Richard (1992) found that female sifakas (*Propithecus verreauxi*) prefer males that act submissively toward them, and it is possible that female spotted hyenas exhibit similar preferences for males that demonstrate high rates of unsolicited appeasement behavior.

We conclude that endurance rivalry does appear to be an important mechanism of male-male competition in spotted hyenas, even though breeding occurs year-round in this species. Based on the data we have presented here, endurance rivalry among male spotted hyenas appears to be a multi-faceted contest, depending on both a male's tenure in the clan and his associations with adult females. Future work should focus on elucidating female preferences in this species and determining what factors affect male reproductive success, with a particular focus on why males that have been in the clan for longer than six years have markedly reduced annual reproductive success compared to males with intermediate tenures.

Acknowledgments

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Figure 3.1. A frequency histogram showing the tenure years during which adult male spotted hyenas (*Crocuta crocuta*) sired their first cubs (gray bars, n=34). The black triangles indicate how many males disappeared during that tenure year without ever having sired any cubs (n=17).

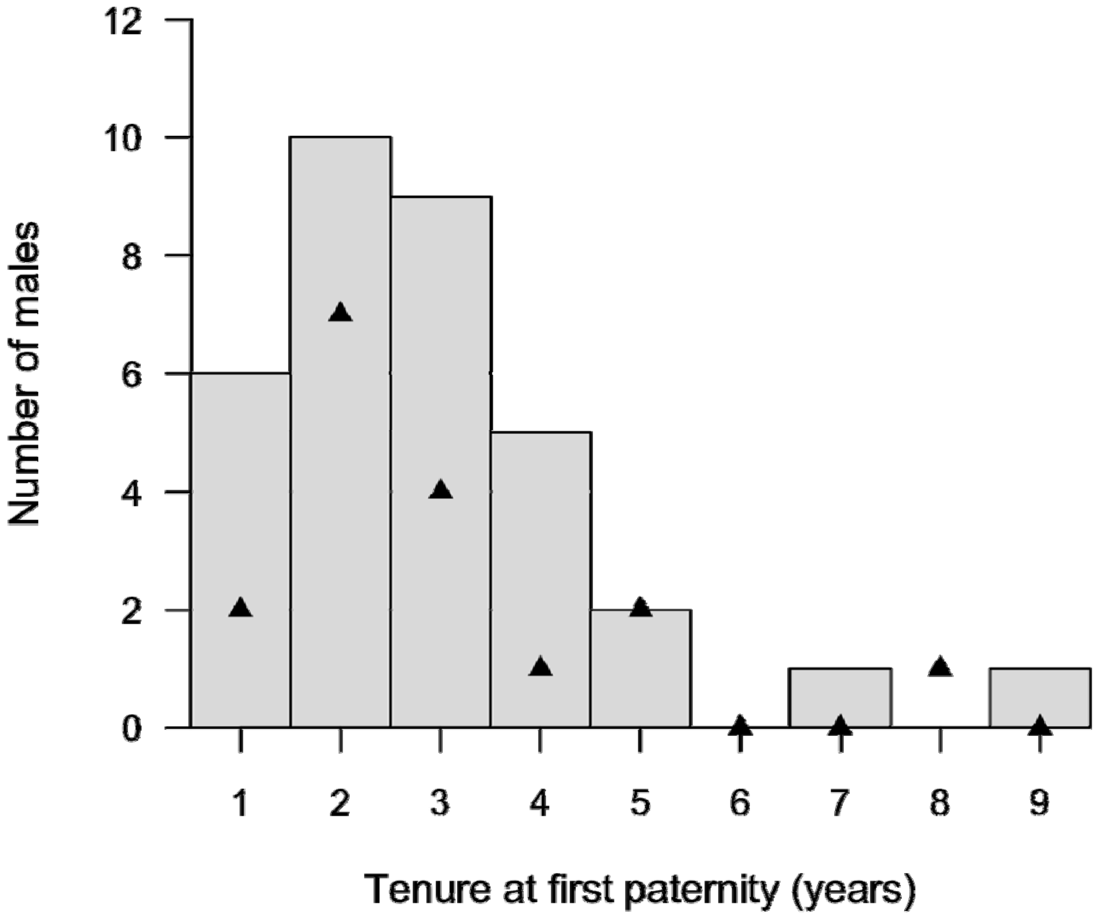

Figure 3.2. A density plot showing the frequency distribution of tenure durations at disappearance among adult male spotted hyenas (*Crocuta crocuta*). The dotted line represents males that never sired any cubs, and the dashed line represents males that sired at least one cub during their tenure in the clan. The two distributions are plotted with equal bandwidth.

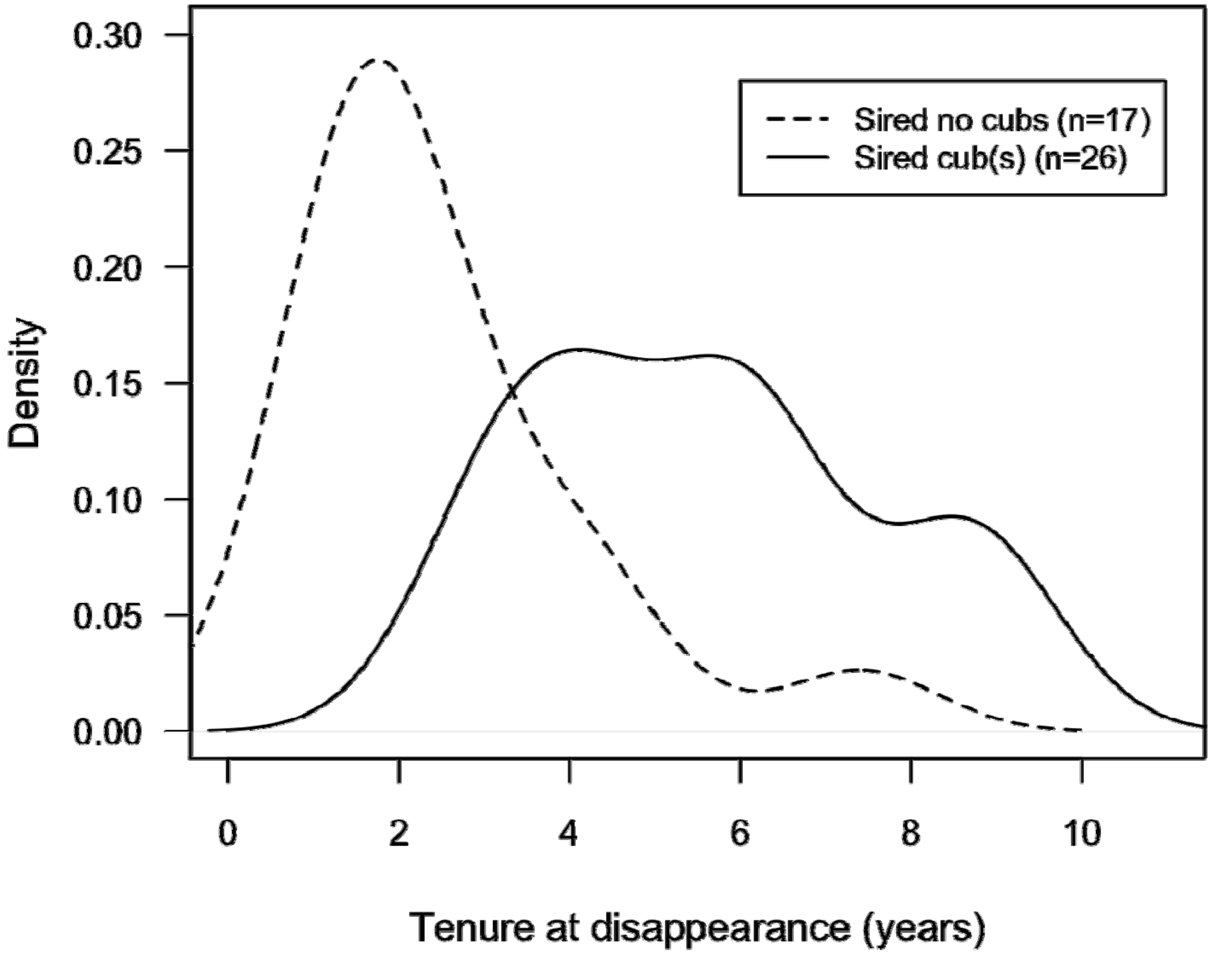

Figure 3.3. The number of cubs an adult male spotted hyena (*Crocuta crocuta*) sired per year as a function of his tenure in the clan. The solid black line represents the posterior mean estimate of the number of cubs a male sired per year generated by the model, and the dotted black lines represent the 95% credible intervals. The gray points represent the raw data used to generate the model. The inset shows a close-up version of the posterior mean estimate and 95% credible intervals, without the inclusion of the raw data, for finer-scale interpretations. Only cubs that reached the age of den graduation were considered in this analysis. Predicted annual RS peaks at tenure = 5.95 years. These results correspond with those from the model presented in Table 3.1.

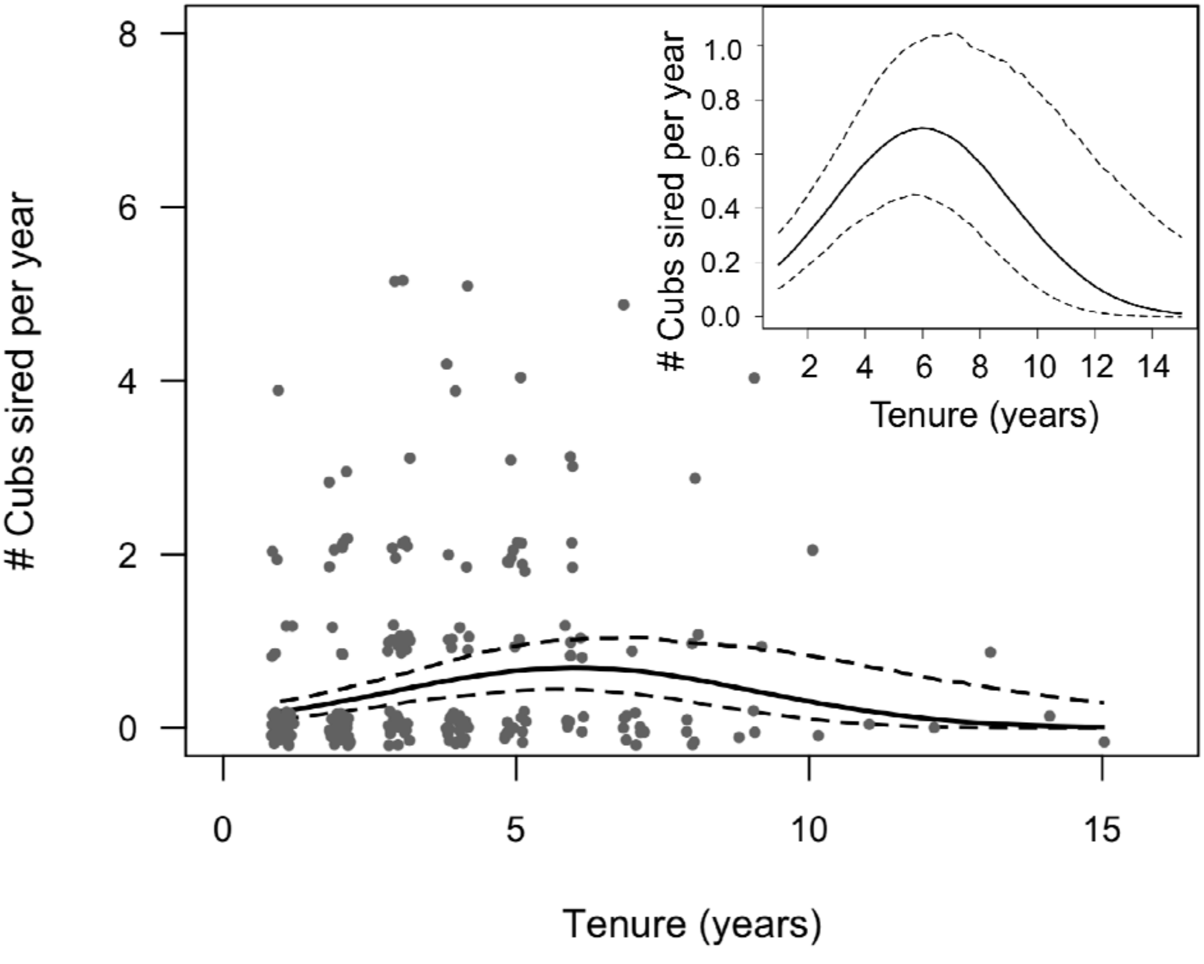

Table 3.1. Outputs of the Bayesian mixed model accounting for variance in annual RS of male spotted hyenas (*Crocuta crocuta*) using MCMCglmm (n=48 hyenas). We report the posterior mean estimates of the coefficients of the fixed effects with 95% credible intervals (CI) and *p*MCMC values. Because the model uses the log-link function, the reported posterior mean estimates are the log-transformed values of the coefficients and represent the number of cubs sired per year relative to the intercept. Fixed effects presented in bold were considered significant because their 95% CIs did not overlap with zero. The random effect is presented with the percent of the total variance it explained. All continuous covariates were standardized prior to their inclusion in the model.

Parameter	Posterior mean (95% CI)	*p*MCMC
Intercept	-0.640 (-1.019, -0.272)	-
Tenure	**0.607 (0.293, 0.937)**	**<0.001**
Tenure^2	**-0.351 (-0.556, -0.123)**	**<0.001**
Mean number of females per session	**0.202 (0.006, 0.439)**	**0.060**
Mean rate of dyadic aggressive acts directed by males toward females	-0.044 (-0.379, 0.218)	0.804
Mean rate of coalitionary aggressive acts directed by males toward females	-0.230 (-0.564, 0.114)	0.192
Mean rate of all aggressive acts received from females	-0.025 (-0.291, 0.256)	0.854
Total number of cubs assigned paternity in the year	**0.410 (0.166, 0.646)**	**<0.001**
Random effect:	Percent of Variance:	
Hyena ID	25.348	-

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CHAPTER 4
DIRECT BENEFITS PROMOTE AGGRESSIVE COALITIONS AMONG MALE SPOTTED HYENAS

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Abstract

Cooperative behavior appears to contradict the predictions of Darwin's theory of evolution by natural selection because altruism benefits a recipient at apparent cost to the donor. Three hypotheses have emerged to resolve this paradox and explain cooperation: kin selection, reciprocal altruism, and direct benefits. Aggressive coalitions have served as useful tools for testing these hypotheses because they are a common form of cooperation among social mammals, but most studies of male coalitions have focused on primates. Here, we investigated aggressive coalitions among males in a non-primate species, the spotted hyena (*Crocuta crocuta*). We found no evidence for kin selection or reciprocal altruism promoting coalition formation among males; instead, male coalitions seemed to be driven by multiple types of direct benefits. First, immigrants utilized coalitions to reinforce the status quo in the social hierarchy. Second, male coalitions were more effective at displacing an individual from a carcass than were dyadic aggressive acts, indicating that male coalitions increase a male's access to food. Third, we found a positive relationship between how often an immigrant participated in coalitions directed against females and his reproductive success, which, given the extreme degree of female choice in this species, suggests the possibility that coalition formation among males may be a trait preferred by females. We did test one hypothesis for a direct benefit that was not supported by our data: we proposed that coalitions might be an effective mechanism for restricting immigration into the clan, which would reduce competition for mates, but male coalitions did not consistently drive out intruder males more successfully than did dyadic aggressive acts.

However, the evidence we found for the three other direct benefits demonstrates that by-product mutualism is the leading force promoting male coalitions in this species.

Keywords: coalitions, cooperation, Crocuta, direct benefits, kin selection, MCMCglmm, reciprocal altruism, spotted hyena

Introduction

In the context of Darwin's (1859) theory of natural selection, which predicts that an individual's behavior should always increase its own fitness, cooperative behavior presents a contradiction because a cooperating individual may act in a way that directly benefits another individual while incurring some cost to itself. In recent decades, several evolutionary forces have been hypothesized to explain cooperation (see West et al. 2007 for review): kin selection (Hamilton 1964; Maynard Smith 1964), reciprocal altruism (Trivers 1971; Axelrod & Hamilton 1981), direct benefits (also referred to as "by-product mutualism"; Brown 1983; Maynard Smith 1983; Connor 1995), and multi-level selection (Wilson 1975; Wilson & Wilson 2007; van Veelen 2009). Aggressive coalitions, in which two or more individuals join in an attack against one or more target individuals (Harcourt 1992), represent a common form of cooperation among social mammals. These complex social interactions bear great potential costs for the cooperators, particularly when the species possesses powerful weaponry, such as teeth specialized for consumption of large prey (Silk 1992; Watts et al. 2006; Olson & Blumstein 2009; Higham & Maestripieri 2010). Given these high potential costs, there must be substantial benefits to participating in an aggressive coalition, which makes aggressive coalitions particularly well suited for testing the hypotheses explaining cooperation (Widdig et al. 2000; Silk et al. 2004).

Among mammals, male-male cooperation is typically less common than female-female cooperation, particularly in female philopatric species in which adult males are generally unrelated (Silk 1994). Nevertheless, mammalian males do cooperate with one another, as has been documented in a number of species (e.g., lions, *Panthera leo*, Packer & Pusey 1982; macaques, *Macaca sylvanus*, Widdig et al. 2000; bottlenose dolphins, *Tursiops truncatus*, Connor et al. 2001; chimpanzees, *Pan troglodytes*, Duffy et al. 2007), and this cooperation often takes the form of aggressive coalitions, hereafter referred to as "male-male coalitions." Male-

male coalitions have been explored extensively in primates (e.g., Packer 1977; Bercovitch 1988; Silk 1992; Silk 1993; Noe 1994; Widdig et al. 2000; Duffy et al. 2007; Bissonnette et al. 2009), but less so in non-primate species (but see Connor et al. 2001; Grinnell et al. 2003; Smith et al. 2010).

Spotted hyenas (*Crocuta crocuta*) present a unique system for using male-male coalitions to explore hypotheses suggesting functional explanations for cooperation. Like many primate species, spotted hyena females are philopatric, with males dispersing from their natal clans after reaching puberty (Smale et al. 1997; Holekamp & Smale 1998). However, whereas many male primates use coalitions to improve their dominance status by joining forces to challenge higher-ranking males (Bercovitch 1988; Bissonnette et al. 2010; Higham & Maestripieri 2010), male hyenas do not appear to use intrasexual aggressive contests to vie for dominance status (East & Hofer 2001). Instead, social rank among male spotted hyenas is based entirely on tenure in the clan, with the longest-tenured male holding the highest rank (Smale et al. 1997; East & Hofer 2001). Furthermore, in stark contrast to most highly gregarious primates, spotted hyenas show striking sex role reversals in that all adult females are socially dominant to all immigrant males (Holekamp & Smale 1998). Despite these unusual social limitations, immigrant male spotted hyenas do form coalitions with one another (Smith et al. 2010). Finally, spotted hyenas are desirable study subjects for investigations into cooperation because they meet many of the criteria typically required for cooperation to occur: they have frequent social interactions (Holekamp et al. 1997; Smith et al. 2007; Smith et al. 2010; Smith et al. 2011), they can recognize third-party relationships (Engh et al. 2005), they have the same potential social partners for many years (Kruuk 1972), and they have varying degrees of genetic relatedness with other members of their clan (Van Horn et al. 2004b).

Our first goal here was to inquire whether association patterns among pairs of immigrant male spotted hyenas change as the relationship between the pair lengthens, and whether there is a correlation between these association patterns and joint participation in coalitions, as has been found in some primates (Silk 1994; Dias et al. 2009; Higham & Maestripieri 2010; Schülke et al. 2010; Berghänel et al. 2011; Kulik et al. 2011). Our second goal was to ask if male-male coalitions directed against adult females were fundamentally different from coalitions directed against other adult targets, as anecdotal evidence has indicated. Specifically, do coalitions against females differ in size or intensity from coalitions against males or intruders of unknown sex? Our third goal, constituting the primary focus of this study, was to explore cooperation among males in a non-primate species by testing three of the non-mutually exclusive hypotheses explaining cooperation: kin selection, reciprocal altruism, and direct benefits.

Predictions of kin selection

According to kin selection theory (Hamilton 1964; Maynard Smith 1964), individuals should cooperate with relatives to gain inclusive fitness benefits. Coalitions of females occur primarily among kin in many social mammals (Chapais et al. 1997; Silk et al. 2004), but in species exhibiting male-biased dispersal, aggressive coalitions among adult male kin are less common (Packer 1977; Bercovitch 1988; Chapais 1995; de Ruiter & Geffen 1998). However, immigrant male dyads in female-philopatric species may vary in their degree of relatedness (de Ruiter & Geffen 1998), and evidence exists supporting the hypothesis that kin selection may be a selective force promoting coalitions among post-dispersal males, in both primates (Chapais et al. 1997; Silk 2002; Silk et al. 2004) and non-primates (Grinnell et al. 2003; Smith et al. 2010).

Spotted hyena males do not disperse in groups, and the mean relatedness among immigrants in a clan has been shown to be approximately zero (Van Horn et al. 2004b), but this does not preclude the possibility of male relatives dispersing to the same clan and having overlapping tenures there. In fact, we know anecdotally of multiple cases in which this has occurred in our study population. Furthermore, spotted hyenas can discriminate between kin and non-kin (Holekamp et al. 1999; Van Horn et al. 2004a; Wahaj et al. 2004), and Smith et al. (2010) found kin selection to be the primary force promoting coalitions among spotted hyena females. It is therefore possible that kin selection may influence an immigrant's participation in male coalitions. This hypothesis predicts that the likelihood of potential immigrant coalition partners participating in a coalition together should increase as their relatedness coefficient increases. Second, if kin selection influences an immigrant's decision to participate in a coalition, the likelihood of an immigrant participating in a coalition should decrease as his relatedness with the target of the aggression increases.

Predictions of reciprocal altruism

The reciprocal altruism hypothesis (Trivers 1971; Axelrod & Hamilton 1981) posits than an individual might behave altruistically toward an unrelated individual with the expectation that the recipient of the help will reciprocate in the future, either by returning that same commodity (e.g., coalitionary support) or a different commodity. Given that the benefit to the initial helper is time-delayed, reciprocal altruism can only occur in species that have long relationships containing repeated interactions with recognizable individuals (Packer 1977; Bercovitch 1988). The relatively advanced degree of intelligence thus required for reciprocal altruism makes it unsurprising that many examples of its occurrence come from primates (e.g., Packer 1977;

Seyfarth & Cheney 1984; Berghänel et al. 2011). However, spotted hyenas also fulfill all of these prerequisites, and have demonstrated cognitive abilities that rival those of some primates (Holekamp et al. 1999; Engh et al. 2005; Holekamp et al. 2007; Drea & Carter 2009; Benson-Amram et al. 2011), so reciprocal altruism is theoretically possible in this species, although Smith et al. (2010) found no evidence for reciprocal altruism among female spotted hyenas. If reciprocal altruism is promoting male coalitions in this species, we would expect to see a positive correlation between how often immigrant A lends coalitionary support to immigrant B and how often B lends support to A.

Predictions of direct benefits

Individuals might act cooperatively because, by doing so, they receive immediate benefits that they could not obtain as effectively by acting alone (Packer & Pusey 1982; Brown 1983; Maynard Smith 1983; Rothstein & Pierotti 1988; Connor 1995). The primary difference between cases of direct benefits and kin selection or reciprocal altruism is that the latter two processes both imply an immediate cost to the donor of the help (Rothstein & Pierotti 1988), whereas in the case of direct benefits, the donor does not incur a net cost. Both the donor and the recipient of help immediately benefit, although not necessarily equally. The type of direct benefit an individual might gain from acting cooperatively can vary widely; here, we tested four hypotheses regarding different direct benefits immigrant male spotted hyenas could gain by participating in male coalitions: 1) male coalitions maintain the status quo of the social hierarchy; 2) male coalitions increase access to food; 3) male coalitions restrict immigration into the clan; and 4) male coalitions attract females. We detail each of these hypotheses below.

Male coalitions maintain the status quo

Immigrant male spotted hyenas appear to utilize neither dyadic nor coalitionary aggression to improve their rank in the social hierarchy (Smale et al. 1997; East & Hofer 2001), most likely because the risk of retaliation is too high (Chapais 1995). We have therefore elected here to test the hypothesis that immigrant males use coalitionary aggression to reinforce their positions relative to lower-ranking individuals (Chapais 1995; Schaik et al. 2004). Immigrants might directly benefit from participating in coalitions that preserve the social order, as has been found in a plethora of species (Bernstein & Ehardt 1985; Silk 1993; Widdig et al. 2000; de Villiers et al. 2003; Silk et al. 2004; Ostner et al. 2008; Berghänel et al. 2010; Kulik et al. 2011), because priority of access to food in this species is determined entirely by social rank (Frank 1986b). Therefore, immigrants of virtually all ranks could benefit from maintaining the status quo, as everyone but the lowest-ranking immigrant has at least one individual that is lower-ranking. If immigrants are using coalitionary aggression to reinforce the social hierarchy, an immigrant should be more likely to participate in a coalition with other males if the target of the coalition is lower-ranking than the immigrant.

Male coalitions improve access to food

Given the intense feeding competition all spotted hyenas experience (Kruuk 1972; Frank 1986b; Smith et al. 2008), immigrant males might participate in aggressive coalitions as a means of displacing competitors from a carcass. Each participant in the coalition would thus directly benefit by increasing its own access to food (Mesterton-Gibbons & Sherratt 2007). This hypothesis predicts that coalitionary aggression should be more effective than dyadic aggression at causing the target of an aggressive to retreat from a carcass.

Male coalitions restrict immigration

Coalitions among male spotted hyenas might also function to restrict potential immigrants from joining the clan. Immigrant males sire 97% of spotted hyena cubs (Engh et al. 2002), so if resident males can decrease the likelihood that an alien intruder will successfully immigrate into the clan, they can effectively limit the pool of potential mates available to sexually receptive females in their clan (Engh et al. 2002). Furthermore, although annual reproductive success generally increases with tenure, longer-tenured males do not monopolize access to females, and shorter-tenured immigrants achieve higher reproductive success than is predicted by the priority of access model (Engh et al. 2002), so new immigrants impose a cost to all resident immigrants in a clan, including those with shorter tenure. Many immigration attempts do not succeed, and this may be in part because alien intruders are met with resistance from the resident males of a clan, who might use aggression to drive aliens away from the clan's territory. Consequently, immigrant males could obtain direct benefits from joining to form coalitions against alien intruders attempting to immigrate into the clan. In fact, previous research on other species has demonstrated that males form coalitions against intruders (Grinnell et al. 2003), even when males do not share territories (Packer & Pusey 1982; Elfström 1997; Backwell & Jennions 2004; Bolton et al. 2011), as do male spotted hyenas.

Male coalitions appeal to female preferences

In many social species, males form coalitions to increase their access to females (Bygott et al. 1979; Bercovitch 1988; Bissonnette et al. 2010), particularly when females are in estrus (Packer 1977; Bercovitch 1988). This can occur even though the cooperating males are typically competing for the same females (Packer & Pusey 1982). In contrast, female spotted hyenas have complete control over copulation (Cunha et al. 2003), so for males, obtaining “access” to females likely means appealing most strongly to female preferences, which are poorly understood. One possibility is that females prefer males that demonstrate cooperative behavior. Male spotted hyenas participate in a unique and highly perplexing behavior known as “baiting” (Szykman et al. 2003), in which multiple adult males cooperatively attack a lone adult female; these attacks represent rare departures from the otherwise strict adherence to the social hierarchy spotted hyenas typically display (Frank 1986b). Although female hyenas are baited most frequently while sexually receptive (Szykman et al. 2003), baiting episodes do not lead to immediate copulations between the baited female and any of the baiting males, so the function of these attacks remains unknown. Szykman et al. (2003) suggested that their function might be to demonstrate some aspect of male fitness to females; perhaps the trait females are cuing into is a male’s ability to cooperate with other males via baiting coalitions. If this hypothesis were correct, we would expect immigrants that participate in more male coalitions against females to have higher reproductive success than immigrants that participate in fewer male coalitions against females.

Methods

Subject population

Our study subjects were members of a wild spotted hyena clan that was continually monitored from 1988-2009 in the Masai Mara National Reserve, Kenya. Over this period, the clan typically contained 50-100 individuals, all of which could be identified by their unique spots and other markings (Frank 1986a). We used the dimorphic morphology of the erect phallus to determine the sex of each individual (Frank et al. 1990), and we estimated birthdates of natal animals using methods described by Holekamp et al. (1996).

Male spotted hyenas typically disperse from their natal clans after reaching puberty at 24 months of age, although this dispersal can be delayed for as long as several years (Smale et al. 1997; Van Horn et al. 2003). A hyena clan therefore contains both adult natal male that have not yet dispersed and immigrant males born in other clans. Adult natal males did not participate in coalitions with other adult males (natal or immigrant) with sufficient frequency to analyze statistically, so we restricted our analyses to coalitions comprised solely of immigrant males. All natal hyenas, male and female, outrank all immigrant males, and among immigrants, rank is determined solely by tenure in the clan, with the longest-tenured immigrant holding the highest rank (Smale et al. 1997; Holekamp & Smale 1998; East & Hofer 2001). We therefore determined an immigrant's social rank using his arrival date in the clan, and confirmed this rank order based on the outcomes of dyadic agonistic interactions (Holekamp & Smale 1993; Smale et al. 1993).

Behavioral data collection and terminology

We conducted observations of the hyenas from vehicles for several hours each morning and evening. Upon encountering a subgroup of one or more hyenas, we began an observation session by recording the identity and activity of the hyena(s) present, including all hyenas within 200m of the subgroup (Smith et al. 2008). We used all-occurrence and scan sampling to record certain behaviors as critical incidents (Altmann 1974), including all aggressive interactions. Aggressive acts involved discrete behaviors assigned to one of two intensity levels: high intensity aggressive acts were bites, bite-shakes, chases, and lunges; low intensity acts were pushes, stand-overs, "points" (aggressive posturing), head waves, and displacements (Smith et al.

2007). Additionally, we recorded whether or not the aggressive contest occurred over a food item, as well as the identity and sex of the target individual, toward which the aggression was directed.

A "dyadic attack" was one that occurred between a single aggressor and a single target, whereas a "coalitionary attack" was one in which multiple aggressors, or "coalition members," cooperated in a joint aggressive attack directed at a single target. In a subset of coalitionary attacks, we were able to determine which coalition member initiated the aggression and which other hyena(s) subsequently joined to lend coalitionary support; in these cases, a joining individual was labeled a "donor" and the initiator was labeled the "recipient," because the latter received the support of the donor. For coalitions in which we could not specifically identify donor(s) and recipient, we simply labeled all aggressors as coalition members, which is still informative, because both donors and recipients can benefit from participating in a coalition (Bercovitch 1988). The number of hyenas participating in a coalition was the "group size," and each coalition was given a unique "coalition ID." When a coalition of immigrants occurred, we considered all immigrants present in the observation session to be "potential cooperators" in that they had the opportunity to participate in the coalition, unless they were the target of the aggressive act. Each potential cooperator then either did or did not participate in the coalition. We restricted our analyses to coalitions in which all members were immigrant males and the target was an adult hyena.

In order to compare male coalitions targeting adult females with those targeting other adults, we assigned each coalitionary act to one of three target groups: resident adult females, resident adult males (both immigrant and adult natal male), and alien intruders (primarily males; Boydston et al. 2001). We then inquired what percentage of coalitionary acts in each target group occurred at high intensity, and compared these percentages using Chi-square tests. We also inquired what percentage of coalitionary acts in each target group had group sizes of three or more (versus a group size of two), and again compared these percentages using Chi-square tests.

Genetic relatedness and analysis of paternity

To collect genetic data, we anesthetized individuals with Telazol (Fort Dodge Animal Health, Fort Dodge, IA, USA, 6.5mg/kg) before collecting blood samples. We then used Puregene kits (Gentra Systems Inc., Minneapolis, MN, USA) to extract DNA from the blood and store it in liquid nitrogen until it could be processed further in the U.S. (Engh et al. 2002). Eleven autosomal microsatellite loci (CCr01, CCr04, CCr05, CCr07, CCr11, CCr12, CCr13, CCr14, CCr15, CCr16, and CCr17; GenBank Accession nos. AY394080-AY394084 and AF180491-AF180497) and one X-linked microsatellite locus (CCrA3; GenBank Accession nos. AY394085-AY394086) were amplified and analyzed using conditions described previously (Libants et al. 2000; Engh et al. 2002). We calculated the Queller-Goodnight *R* relatedness estimates between pairs of immigrant males using the program Coancestry (Queller & Goodnight 1989; Wang 2010). Only individuals that had been genotyped at more than eight loci were included in the Coancestry relatedness analysis.

To assess paternity, we first calculated the date of conception for each natal animal by subtracting 110 days, the known gestation period (Holekamp et al. 1996), from the animal's estimated birthdate. We then calculated a conception period ranging from six months before, to two months after, the estimated date of conception as a conservative compensation for errors in birthdate estimations. Any immigrant, transient, or unrelated adult natal male present in the clan during this conception period was considered a potential father for that offspring. A natal male was classified as an adult if it was at least 36 months of age by the end of the conception period, and was deemed unrelated when its coefficient of relatedness to the mother of the offspring, calculated from known maternal genealogies, was <0.125. We then used the program CERVUS to assign paternity to offspring (Marshall et al. 1998), using the following input parameters: number of candidate fathers=25, 90% of candidate fathers sampled, 88% of loci typed, and an error rate of 1%. Only individuals that had been typed at a minimum of six loci were included in the analysis. We considered a male to be the father of an offspring when CERVUS assigned him the paternity with 95% confidence.

Statistical modeling and reporting

Most of our analyses relied on Bayesian Markov Chain Monte Carlo generalized linear mixed models using the R package MCMCglmm (Hadfield 2010). MCMCglmm is a particularly useful Bayesian tool because of its suitability for non-parametric data and its flexibility regarding random effects. For specifications on input parameters for the models, such as the priors, iterations, and thinning intervals used, see Appendix C. We report the posterior mean estimates of coefficients for all fixed effects, as well as the 95% credible intervals (CI) and corresponding p-values. The models utilized several different link functions, so in the caption for Table 4.1, we have included specific instructions on the back transformations required to interpret the absolute values of the coefficients for each model. In models containing categorical fixed effects, we set the reference level of the model at the value with the highest sample size for each categorical fixed effect; in our reported results, the intercept represents this reference level, as well as the mean for all continuous fixed effects. All posterior mean estimates of the coefficients for each parameter are then presented relative to the reference level (intercept). A fixed effect was considered significant when its 95% CI did not overlap with zero. Additionally, we report the proportion of variance explained by each random effect relative to the total variance, expressed as a percentage. All statistical analyses were carried out in R v. 2.13.0 (R Development Core Team 2011). All other means are reported with the standard error of the mean unless otherwise noted.

Association index calculations and link to coalition participation

We used the twice-weight association index of Cairns & Schwager (1987) to evaluate affiliation among pairs of immigrant males, as was done previously in studies on spotted hyenas (Holekamp et al. 1997; Szykman et al. 2001; Smith et al. 2007). The association index for any pair of individuals A and B ($AI_{A,B}$) was calculated as $(A+B_{together})/[(A_{without\ B})+(B_{without\ A})+(A+B_{together})$, where $A+B_{together}$ represents the number of sessions in which A and B were both present, $A_{without\ B}$ was the number of sessions in which A was present but B was not, and $B_{without\ A}$ was the number of sessions in which B was present but A was not. To be included in our analysis, a pair had to have been concurrently present in the clan for at least six months; the total amount of time each pair overlapped is hereafter referred to as that pair's "relationship length." We first calculated an AI for a pair's entire relationship length and termed this the pair's "lifetime AI." Then, because we were interested in how affiliation patterns changed over the course of a relationship, we divided each pair's relationship length into six-month periods, beginning with the first date on which both individuals were present concurrently in the clan. We then calculated an association index (AI) for each unique dyad/period combination. Only periods in which both individuals were seen at least ten times during the period were included in the analysis. We also excluded periods occurring nine years or more into a relationship because our sample sizes for those periods were miniscule.

To describe how affiliation patterns within each pair of immigrants changed over the course of the relationship, we plotted median values for each period across all pairs whose relationship extended to that length. We then statistically evaluated how affiliation patterns change over time by modeling the effects of a single fixed effect, the six-month period in the relationship (months 0-6=period 1, months 7-12=period 2, etc.), on a pair's AI for that period (model_{AI}). We included individual identity (ID) and pair ID as random effects because we had repeated measures for individual males across multiple pairs and repeated measures for pairs across multiple periods. We log-transformed the response variable (a pair's AI for a given period), and we standardized period to have a zero mean and unit variance before including it in the model; all other standardizations in this study were performed in the same way. Finally, if a given dyad was never observed together during a specific time period, that may reflect a real biological phenomenon pertinent to the relationship, but it may also have been due to sampling error. We therefore restricted this model to periods in which a dyad was seen together at least once (i.e., their AI for that period was greater than zero).

We used a similar model to assess whether association patterns were correlated with joint participation in male coalitions (model_{AC}). In this model, however, we used a binomial response variable describing how many times a pair of immigrants participated in a coalition together when they had opportunities to do so (i.e., both were present when the coalition occurred), and how many times they did not participate in a coalition together, with the sum of those two response variables equaling the total number of opportunities the pair had to participate in the same coalition. The fixed effect was the pair's standardized lifetime. Finally, in order to be able to include a random effect for hyena ID, we created a duplicate of each data point (pair) and assigned one of the data points to one member of the pair, and the second point to the other member of the pair, and then corrected for this duplication by including a random effect of pair.

Testing the kin selection hypothesis

Model$_{KS}$ explored how a potential cooperator's relatedness to both the target of a coalitionary attack and to the other member(s) of the coalition affected the probability that the potential cooperator would participate in that coalition. If a potential cooperator did not ultimately participate in a coalition, there would be multiple R-values: one between the potential cooperator and each of the coalition members. Likewise, there would also be multiple R-values if the coalition group size was >2, regardless of whether the potential cooperator ultimately cooperated or not. In these cases, we used the maximum R-value for a potential cooperator (R_M). We elected to use the maximum R-value and not the average R-value because including both introduced substantial multicollinearity into the model, and we felt that the maximum R was the more biologically salient value. If a male were electing to join a coalition to lend support to a specific individual with which it shared a high proportion of genes, this effect could be masked if there were multiple unrelated individuals also participating in the coalition, which would decrease the average R.

The fixed effects in the model were then R_M and the potential cooperator's R with the target (R_T), with a binary response variable indicating whether or not the potential cooperator participated in the coalition. We also included the sex of the target as a fixed effect, and the following interaction terms: target sex x R_M and target sex x R_T. The reference level of model$_{KS}$

was an immigrant target. Potential cooperator ID, target ID, coalition ID, and session number were all included as random effects in the model. R_M and R_T were both standardized before their inclusion in the model.

We limited our analysis to coalitions directed against adult females and other immigrants; there were so few coalitions directed against adult natal males that their inclusion introduced high levels of multicollinearity. Likewise, we only included coalitions in which we had genetic data for the target of the aggression, and we excluded any potential cooperators for whom we did not have genetic data. Finally, we did not include any coalitions against aliens in this analysis because we lacked genetic data for them.

Testing the reciprocal altruism hypothesis

To test for the predicted correlation between the amount of coalitionary support immigrant A donated to immigrant B and the amount of support A received from B, we used partial rowwise matrix correlation methods (Hemelrijk 1990b; Hemelrijk 1990a; De Vries 1993). This approach necessitated limiting the dataset to include only those coalitions in which we could identify a clear immigrant donor and a single immigrant recipient. Additionally, only immigrants that either benefited from support from another immigrant or donated support to another immigrant at least once were included in the matrix (Hemelrijk 1990a). We analyzed our composite dataset, which included data from the entire study period (1988-2009), as well as three subsets of data from shorter periods (1990-1994, 1996-1999, and 2001-2006).

Rather than using the absolute number of times A helped B, which would not correct for the number of opportunities A had to lend support to B, we converted the data to a proportion: the number of times A helped B divided by the number of opportunities A had to lend support to

B. We defined an “opportunity to lend support” as any instance in which A was present when B initiated a dyadic aggression against a third party. To distinguish between true zeros (A had at least one opportunity to help B, but never lent support) and structural zeros (A did not have any opportunities to help B), we used a complementary matrix that indicated whether or not A had at least one opportunity to help B. To perform these analyses, we used the software program MATMAN 1.0 (Noldus Information Technologies, Wageningen, the Netherlands; De Vries et al. 1993).

Testing the direct benefits hypothesis

Model$_{SQ}$ tested the predictions of the status quo hypothesis by modeling the probability of a potential cooperator participating in a coalition based on a single fixed effect: whether or not the potential cooperator was higher-ranking than the target of the aggressive act. Because all adult females are higher-ranking than all immigrant males, male coalitions directed toward adult females cannot, by definition, be reinforcing the status quo. Other adult males, however, can be either higher- or lower-ranking, so this hypothesis remains viable for male coalitions with male targets. We therefore restricted this model to coalitions directed against other adult males. The reference level of model$_{SQ}$ was the potential cooperator being higher-ranking than the target. As in model$_{KS}$, we included potential cooperator ID, target ID, coalition ID, and session number as random effects.

To test the hypothesis that coalitions provide increased access to food, we used a model, model$_{FA}$, to compare the efficacy of aggressive coalitions among immigrants with the efficacy of dyadic aggression directed from one immigrant toward another in conflicts over food. An aggressive act occurring over food was deemed effective when the target of the aggressive act retreated from the food, and a target was considered to have retreated if it was not feeding in the first scan occurring immediately after the contest. If no scan was performed within ten minutes, that aggressive act was excluded from the analysis. Likewise, when a target received multiple aggressive acts in succession without a scan performed between them, regardless of the identity of the aggressor (immigrant or otherwise), we included only the final aggressive act, and only if the aggressor was an immigrant. Model_{FA} modeled whether or not a target retreated from the food item as a function of whether the aggressive act was coalitionary or dyadic, with dyadic aggression being the reference level. For coalitions, we included a separate record for each member of the coalition so we could control for individual differences in how likely a particular aggressor was to elicit a retreat from a target. We accomplished this by including aggressor ID as a random effect; we also included target ID as a random effect. Finally, because 95.5% of aggressive acts occurring over food were directed against males, we excluded the few aggressive acts directed by immigrants against females, because their inclusion introduced high multicollinearity.

Our analysis testing the hypothesis that coalitions restrict immigration was very similar to our analysis testing the food access hypothesis. In this case, model_{RI} compared the efficacy of coalitions with that of dyadic aggressive acts. In this case, however, we only examined coalitions directed against alien individuals, and the response variable was whether or not the alien target retreated and left the session. An alien was considered to have retreated if it met one of the following criteria: it was more than 100m from the focal group at the time of the first scan immediately after the aggressive act, it was leaving the session at the time of this scan, or it had left the session prior to this scan. As in model_{FA}, if no scan was performed within ten minutes, we excluded the preceding aggressive act from our analysis, and when an alien received multiple aggressive acts without a scan performed between them, we included only the final aggressive act, and only if the aggressor was an immigrant. Model$_{\text{RI}}$ then asked if an alien's probability of retreating differed if it was the target of an aggressive coalition versus a dyadic aggressive act. Furthermore, we included a fixed effect term describing whether or not the alien ultimately went on to join the clan or nor, and an interaction between this term and whether the aggressive act was dyadic or coalitionary. The reference level of model$_{\text{RI}}$ was dyadic aggression directed toward an alien that did eventually join the clan. Once again, we included a separate record for each member of a coalition and then included aggressor ID as a random effect.

We tested the hypothesis that immigrant coalitions provide direct benefits through female preference for males that bait by modeling an immigrant's lifetime reproductive success in the clan as a function of his lifetime participation in baitings (i.e., coalitions in which the target of the attack was an adult female). To measure lifetime reproductive success, we counted how many cubs an immigrant was known to have sired throughout his entire tenure in the clan using the paternity analysis explained above. To measure lifetime participation in coalitions against females, we counted how many times throughout his tenure in the clan an immigrant participated in baiting episodes. We then divided this number by the number of opportunities he had to participate and expressed his lifetime participation as a percent. We limited our analyses to immigrants who had a minimum of five opportunities throughout their lifetime in the clan. The response variable in this model, model$_{\text{RS}}$, was the total number of cubs an immigrant sired, and the primary fixed effect was his lifetime participation in baiting episodes (%). However, because we know that lifetime reproductive success for immigrants in this species increases as total tenure increases (Engh et al. 2002), we also included an immigrant's total tenure in the clan as a fixed effect, as well as the interaction term tenure*% participation in baiting episodes. Both lifetime tenure and % participation were standardized prior to their inclusion in the model.

Results

Coalitions among immigrant males

From 1988-2009, 94 resident male hyenas participated in 502 distinct male-male coalitions directed against adults. Because adult natal males participated in only 24.1% of these, we excluded them from all analyses, and only examined coalitions made up exclusively of immigrant males. Of these 381 coalitions, there were 57 individual participants, 38.32% were at high intensity, and the mean group size was 2.42±0.04. 52.23% of coalitions were directed against resident adult females, 41.21% against adult males, and 6.56% against alien individuals of unknown sex. The proportion of coalitions that were at high intensity was significantly less when the target was a resident female (29.15%) than when the target was a resident male (43.33%) or alien (80.00%; Figure 4.1; χ^2=27.12, df=2, p<0.001). The proportion of coalitions that consisted of three or more members was significantly greater when the target was a resident female (41.21%) than when the target was a resident male (21.02%) or alien (24.00%; Figure 4.2; χ^2=17.25, df=2, p<0.001). In light of these qualitative differences, we separated coalitions against females from coalitions against males and aliens in many of our subsequent analyses.

Genetic relatedness and paternity analysis

Among the 69 immigrants that had opportunities to participate in coalitions with other immigrants, we obtained genetic data from 52 individuals. The mean pairwise Queller-

Goodnight *R* among these 52 immigrants (n=1326 pairs) was 0.027±0.004. Of the 250 offspring included in the paternity analysis, we were able to assign paternity to 176 (70.4%), 172 (97.7%) of which had known, genotyped mothers. The mean observed error rate across all loci was 2.5%.

Association indices among immigrant males and link to coalition participation

The mean association index across all blocks for all pairs (n=4996 pair-blocks) was 0.060±0.001. After excluding the pair-blocks with AI=0, the remaining 4186 pair-blocks had a mean of 0.072±0.001, which included data from 624 unique pairs formed among 74 immigrant males. Both Figure 4.3 (which includes zero values of AIs) and the results of model_{AI} (which excludes zero values of AIs; Table 4.1) demonstrated that association indices among male pairs increased significantly as the relationship lengthened. The complete results of this model and all subsequent models, including posterior mean estimates for each fixed effect with corresponding credible intervals and p-values, are shown in Table 4.1. Model_{AC}, which assessed 59 hyenas in 431 unique pairs, revealed a significant positive relationship between a pair's lifetime AI and how often that pair participated in coalitions together (Table 4.1). The mean lifetime AI for the 374 pairs analyzed in model_{AC} was 0.065±0.001.

Kin selection

To test the kin selection hypothesis, model_{KS} (Table 4.1) explored the relationship between a potential cooperator's relatedness to the coalition member(s) or the target of the aggression and his probability of participating in that coalition. This model included 52 individual potential cooperators, 71 individual targets, 181 sessions, and 317 distinct coalitions.

There was no significant effect of the relatedness to the target (R_T), regardless of whether the target was a female or an immigrant male. Similarly, there was no effect of maximum relatedness (R_M) when the target was female. However, when the target was an immigrant male, the probability of a potential cooperator participating in a coalition significantly decreased as R_M increased (Figure 4.4), which is in direct contradiction to what the kin selection hypothesis would predict.

Reciprocal altruism

We used partial rowwise matrix correlation tests to test for a positive correlation between how much coalitionary support an individual immigrant gave another immigrant and how much he received from that same partner. After controlling for whether or not a pair of immigrants had opportunities to lend support to one another, we found a small but significant positive correlation between support given and support received over the course of our entire study period (*TauKr*=0.067, *p*=0.019; Table 4.2). However, when we performed the same analysis on data from three shorter time spans containing fewer structural zeros, two had non-significant correlation coefficient, and one had a significant negative correlation coefficient; in other words, none of the three indicated reciprocal altruism was occurring.

Direct benefits

Model_{SQ} (Table 4.1) tested the status quo hypothesis by investigating whether the relative rank of the target affected a potential cooperator's probability of participating in a coalition by examining 57 individual potential cooperators, 47 individual male targets, 102 sessions, and 145 distinct coalitions. Immigrant males were significantly more likely to participate in a coalition against other males if they were higher-ranking than the target of the aggression than if they were lower-ranking than the target (Figure 4.5).

To test the hypothesis that coalitions provide increased access to food, model_{FA} (Table 4.1) compared the effectiveness of coalitionary versus dyadic aggression at displacing the target of the aggression from a food item. This model contained 45 individual aggressors and 65 individual targets. Coalitionary aggression was significantly more likely to result in the target retreating from the food item (81.3% of coalitions resulted in retreat) than was dyadic aggression (50.6% of dyadic aggressive acts resulted in retreat).

Model_{RI} (Table 4.1) tested the hypothesis that coalitions restrict immigrant by comparing the effectiveness of coalitionary versus dyadic aggression at driving away alien individuals that might have been trying to join the clan as new immigrants; there were 46 individual aggressors included in this model. Coalitionary aggression was significantly more likely to result in the alien individual retreating from the session than was dyadic aggression when the alien went on to ultimately join the clan. However, in cases in which the alien did not ultimately join the clan, there was no difference between dyadic and coalitionary aggression in the likelihood that the alien would retreat.

We tested the hypothesis that coalitions appeal to female preferences with our final model, model_{RS} (Table 4.1). This model explored a link between an immigrant's lifetime participation in baiting episodes against females and his lifetime reproductive success in the clan. Lifetime participation in baiting episodes had a significant positive association with the total number of cubs an immigrant sired during his tenure in the clan (Figure 4.6). We also found that an immigrant's total tenure in the clan significantly increased the total number of cubs he sired, but we found no significant interaction between lifetime tenure and lifetime participation in baiting episodes.

Discussion

Target sex affects coalition size and intensity

Male coalitions against resident female targets were significantly larger (Figure 4.2) and significantly less intense (Figure 4.1) than coalitions against males or against alien individuals. Although coalition size has been linked to efficacy in other species (e.g., Bygott et al. 1979), such a link is difficult to assess in spotted hyenas at this time because the precise function of baiting episodes remains unknown (Szykman et al. 2003). The larger mean group size for coalitions against females might be explained by a female preference for being baited by larger coalitions, but it is also possible that male spotted hyenas bait in larger groups because of stress that is potentially induced by the interaction. Baiting episodes represent a complete social role reversal, and are the only circumstances under which adult females are observed to behave submissively toward males (Holekamp & Smale 1998; Szykman et al. 2003). Furthermore, females often retaliate against males during baiting episodes, so males risk injury by participating. Therefore, perhaps a male's perception of these risks decreases as coalition size increases. Similar reasoning can be applied to the difference in intensity level that we observed between coalitions against female targets and coalitions against males or aliens. Coalitions against females were of significantly lower intensity, which may reflect the conflicting motivations immigrants experience regarding participating in a baiting episode.

Immigrant relationships correlate with coalition participation

As their relationships lengthened, pairs of immigrant male spotted hyenas associated more closely (Figure 4.3, Table 4.1). Furthermore, we found that the genetic relatedness between two males had no effect on their likelihood of participating in a coalition together, but there was a significant positive correlation between this likelihood and their lifetime AI (Table 4.1). These results resemble those obtained from macaques showing similar links between male sociality and coalition participation: male macaques with stronger bonds have repeatedly been shown to form more coalitions together than males with weaker social bonds (Silk 1994; Higham & Maestripieri 2010; Schülke et al. 2010; Berghänel et al. 2011; Kulik et al. 2011). Additionally, the absolute values of lifetime AIs among immigrant males that we found here closely match the values earlier studies have found among male-female pairs (Szykman et al. 2001) and pairs of unrelated females (Holekamp et al. 1997).

No evidence of kin selection in male coalition formation

Our data did not support the hypothesis that kin selection favors coalition formation among immigrant male spotted hyenas, as the likelihood of a male participating in a male coalition actually decreased as his maximum relatedness to coalition members increased (Table 4.1, Figure 4.4). There was also no relationship between a male's relatedness to the target of the coalition and his likelihood of participating, which would have been consistent with the kin selection hypothesis. These results align with research showing that kin selection is not the primary force driving cooperation among male lions (Packer & Pusey 1982). Furthermore, Kulik et al. (2011) found that although male rhesus macaques (*Macaca mulatta*) often have close kin available as potential coalition partners, they do not preferentially form coalitions with these kin.

Immigrant male spotted hyenas are solitary dispersers and therefore do not typically have close kin available as social partners, but our data and previous data demonstrate that there is variation in the genetic relatedness among immigrants (Van Horn et al. 2004b). Thus, the potential for kin selection does exist, although we saw no evidence for it here. These results contrast with those found for female spotted hyenas by Smith et al. (2010), which demonstrated that kin selection strongly favors coalition formation among females in this species. This contrast is unsurprising, however, given that female hyenas are philopatric and regularly have close kin available as social partners.

It is curious that immigrants were actually *less* likely to participate in male coalitions as their maximum relatedness to the other members of the coalition increased (Figure 4.4). This may be evidence for kin competition, such as that exhibited by male rhesus macaques, in which males intervene in conflicts more frequently when close kin are involved, but bias their support away from their kin (Kulik et al. 2011). However, more research is necessary before we can draw this conclusion.

No evidence of reciprocity in coalitionary support exchanged by immigrant males

Although our broadest matrix, encompassing all the immigrants in our dataset and spanning 22 years (Table 4.2), did show a significant positive correlation between the number of times immigrant A donated coalitionary support to immigrant B and the number of times A received support from B, we do not consider this to be enough evidence to conclude that reciprocal altruism is operating in this case. For one, although the correlation coefficient was statistically significant, its value was close to zero, implying that even if the coefficient represents a “true” relationship, it is an extremely weak force and likely has little impact on the decision-making of immigrant males regarding when to lend coalitionary support (Widdig et al. 2000). Second, when we examined three smaller matrices spanning shorter time spans (Table 4.2), we found no evidence of reciprocity.

There are multiple possible explanations for these results. First, it is possible that immigrant males actually do exchange coalitionary support, but we do not observe these interactions often enough to detect this reciprocity (Silk et al. 2004). Alternatively, perhaps immigrants exchange coalitionary support for a different commodity that we did not explore here, such as social greetings or feeding tolerance (Smith et al. 2011). The most likely explanation, however, is that immigrant males may simply interact too infrequently for social commodities to be effectively exchanged (Trivers 1971). Most examples of reciprocity occur on shorter time scales than we analyzed here (Barrett & Henzi 2002), and it is possible that there are not enough opportunities for a donor to benefit from reciprocated help within the temporal confines of a hyena's cognitive capacity. Supporting this notion is the fact that examples of pure reciprocal altruism are rare in the non-human animal literature (Clutton-Brock 2002), and also that similar tests for reciprocal altruism in coalition formation have likewise failed to find evidence for reciprocity (Grinnell et al. 2003; Silk et al. 2004), including among female spotted hyenas (Smith et al. 2010).

Evidence for immigrants directly benefitting from participating in male coalitions

We tested four different hypotheses suggesting direct benefits that immigrant male spotted hyenas might accrue by participating in aggressive coalitions with other immigrants. The evidence supported three of the four hypotheses, indicating that by-product mutualism is a strong force promoting cooperation among immigrants.

The first direct benefit we explored was the hypothesis that male coalitions reinforce the status quo. Immigrants were most likely to participate in a coalition against another male if they were higher-ranking than the target of the coalition (Table 4.1, Figure 4.5), which suggests that they utilize coalitionary aggression to maintain the social hierarchy rather than challenge it. The data presented here align with previous research demonstrating that female spotted hyenas also use coalitions to reinforce the status quo (Smith et al. 2010), and with trends observed in macaques, in which males rarely form "revolutionary" coalitions against higher-ranking targets (Bernstein & Ehardt 1985; Silk 1993; Widdig et al. 2000; Ostner et al. 2008; Berghänel et al. 2010; Kulik et al. 2011).

These results are somewhat difficult to interpret, however, because the status quo hypothesis is, to a degree, inherently circular: as males reinforce the status quo by directing aggression down the hierarchy, the hierarchy becomes more stable, and as the hierarchy becomes more stable, males become less likely to challenge it by directing aggression up the hierarchy. The potential costs associated with the risk of retaliation from more powerful higher-ranking males have been cited as reasons why revolutionary coalitions might be rare in some primates (Chapais 1995; Widdig et al. 2000). This explanation seems less applicable to spotted hyenas, however, because male rank is not at all associated with fighting ability in this species (East & Hofer 2001), so the combined fighting ability of two lower-ranking males should almost always be able to exceed that of a lone higher-ranking target. In spite of this, attempts by male hyenas to increase their social rank via physical contests of any kind (dyadic or coalitionary) are extremely rare (East & Hofer 2001).

Next, we asked whether coalitionary aggression was more effective than dyadic aggression at displacing a competitor from a carcass, thereby providing the coalition members with increased access to food because there is one fewer individual at the carcass which to have to share (Mesterton-Gibbons & Sherratt 2007). Male coalitions resulted in the target retreating significantly more often than did dyadic aggressive acts by males (Table 4.1), indicating that males do indeed seem to receive the direct benefit of increased food access when they form coalitions. This result is particularly intriguing because it contrasts with the findings of Smith et al. (2010), in which coalitions of adult female spotted hyenas were no more effective at driving a female competitor away from a carcass (52.6%) than were adult females acting alone (51.6%). These percentages match our estimate of how frequently dyadic male aggression results in retreat by male targets (50.8%), but are significantly lower than our estimate of how often coalitionary male aggression results in retreat by male targets (81.3%). Smith et al. speculated that coalitions might not provide additional benefits beyond those provided by dyadic aggression because the opportunity costs may be too great; when feeding competition is intense, as it often is among spotted hyenas (Kruuk 1972; Frank 1986b), any time allocated away from feeding is likely to translate directly into reduced food intake.

Why, then, would coalitions of males be so much more effective at displacing competitors from a carcass than coalitions of females? Smith et al. (2010) exclusively examined female coalitions against female targets, whereas we examined male coalitions against male targets. It is possible that the difference in efficacy lies in some quality inherent in coalitions of each sex, but it is equally likely that the difference comes from the behavior of the targets. The way males perceive coalitionary aggression over food may fundamentally differ from how females perceive it, although this is not a hypothesis we can currently test.

In contrast, our results did not support the hypothesis that coalitionary aggression restricts immigration. Had aliens that did not go on to immigrate into the clan retreated more in response to coalitionary aggression than they did in response to dyadic aggression, the data would have supported our hypothesis. We found the opposite effect, however: these aliens showed no significant difference in their response to dyadic versus coalitionary aggression (Table 4.1). Interestingly, however, aliens that did ultimately immigrate into the clan retreated significantly more often after receiving coalitionary aggression than they did after receiving dyadic aggression (Table 4.1). Perhaps there is an unknown factor at work that affects both an alien's likelihood of retreating in response to aggression and his likelihood of joining a clan; for example, perhaps immigration success is related to a male's ability to behave in a "socially appropriate" way, and retreating from coalitionary aggression is one way to demonstrate that ability to resident clan members.

The final direct benefits hypothesis we tested suggested that male coalitions appeal to female preferences. Immigrants that took advantage of a greater proportion of baiting opportunities over the course of their tenure in the clan had significantly higher paternity success during this period than did males that participated in relatively fewer baiting episodes (Table 4.1, Figure 4.6), which is consistent with this hypothesis. Because we used observational data and did not directly test female choice experimentally, we cannot conclusively ascribe the correlation between baiting participation and reproductive success to "female choice." However, given the extreme degree of female control over copulation in this species (Cunha et al. 2003), and the fact that the precise function of baiting is unknown (Szykman et al. 2003), Szykman et al.'s (2003) suggestion that baiting may be a form of courtship display remains a viable one, and the data we have presented here, linking baiting participation to male reproductive success, are consistent with that hypothesis.

Precisely why females might prefer males that participate in proportionally more baiting episodes remains unclear. Male-male cooperation may serve as an indicator of male quality to females: coalitionary aggression in any context, including baiting episodes, requires some degree of behavioral synchrony (Szykman et al. 2003), which has been shown in other species to be preferred by females as a potential signal of male quality (Nuechterlein & Storer 1982; Trainer & McDonald 1993; Backwell et al. 1999; Connor et al. 2006). Alternatively, perhaps there are specific, nuanced behaviors exhibited by individual males within a baiting episode that the target female evaluates, such as which male initiates each antagonistic interaction within the baiting episode, or how each male responds to the female's behavior during the episode. Future work should explore these possibilities by examining baiting behavior at a finer scale than has been done previously.

Regardless, this unusual preference for male-male cooperative behavior may be related to the sex role-reversed nature of spotted hyena societies. In species with traditional sex roles, females often show preferences for males that succeed in combat against other males (Andersson 1994). In this role-reversed species, the significance of male-male combat with regard to female preferences is presumably reduced; whether this reduction actually causes, or simply allows for, the increased significance of less agonistic characteristics, such as cooperative behavior, remains undetermined.

Conclusion

The data we have presented here clearly indicate that associations among immigrant male spotted hyenas strengthen over the course of a pair's relationships, that the overall strength of that association predicts how often a pair will cooperate, and that in general, immigrants utilize cooperative coalitions to opportunistically gain direct benefits for themselves. We found no evidence for kin selection or reciprocal altruism favoring the formation of male coalitions, but we found multiple lines of evidence supporting the direct benefits hypothesis, similar to what Grinnell et al.'s (2003) findings in lions. The comparisons between our results and those of Smith et al. (2010), which explored coalitions among female hyenas, highlight the differences between the sexes regarding the evolutionary forces promoting cooperation. Whereas we found coalitions among males to be driven by direct benefits, with kin selection playing a minimal role, kin selection has been shown to be the driving force favoring coalitions among females. Neither males nor females appear to exhibit reciprocal altruism, and both sexes primarily use coalitions to maintain the status quo rather than to challenge it, but only males benefit from forming coalitions by improving their access to food. Finally, the benefits a male derives from cooperating extend directly to his reproductive output, because our data indicate that males may use coalitions to appeal to female preferences. In sum, our results indicate that aggressive coalitions of immigrant male spotted hyenas are driven by opportunistic pursuits of direct benefits, rather than by alternative evolutionary forces that can favor coalition formation.

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Figure 4.1. Barplots showing what percent of 381 coalitions formed by immigrant male spotted hyenas (*Crocuta crocuta*) occurred at high intensity against each of three target types. Error bars represent 95% confidence intervals. Letters above the bars indicate significant differences between the groups (χ^2=27.12, df=2, *p*<0.001).

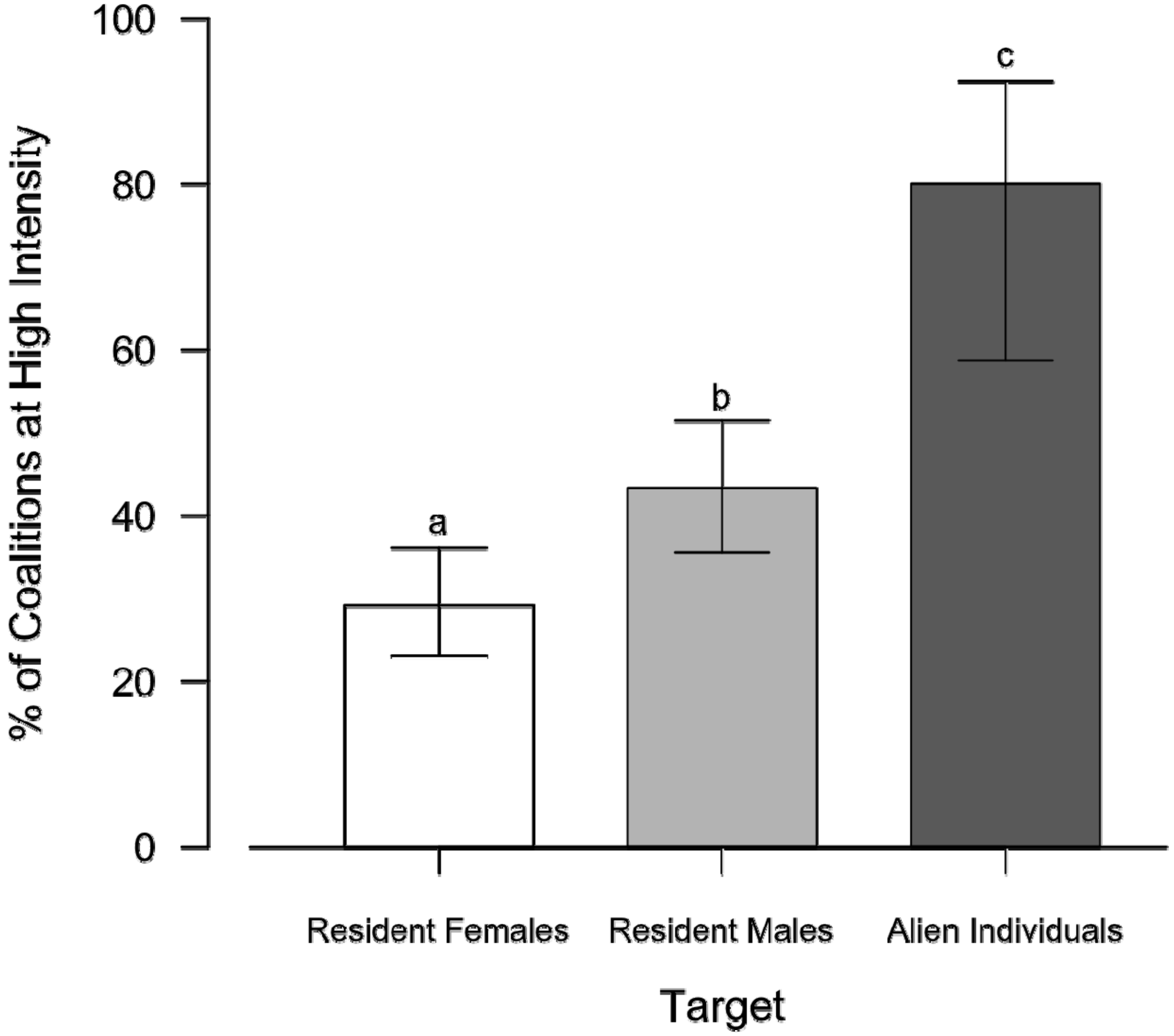

Figure 4.2. Barplots showing what percent of 381 coalitions formed by immigrant male spotted hyenas (*Crocuta crocuta*) consisted of three or more coalition members against each of three target types. Error bars represent 95% confidence intervals. Letters above the bars indicate significant differences between the groups (χ^2=17.25, df=2, p<0.001).

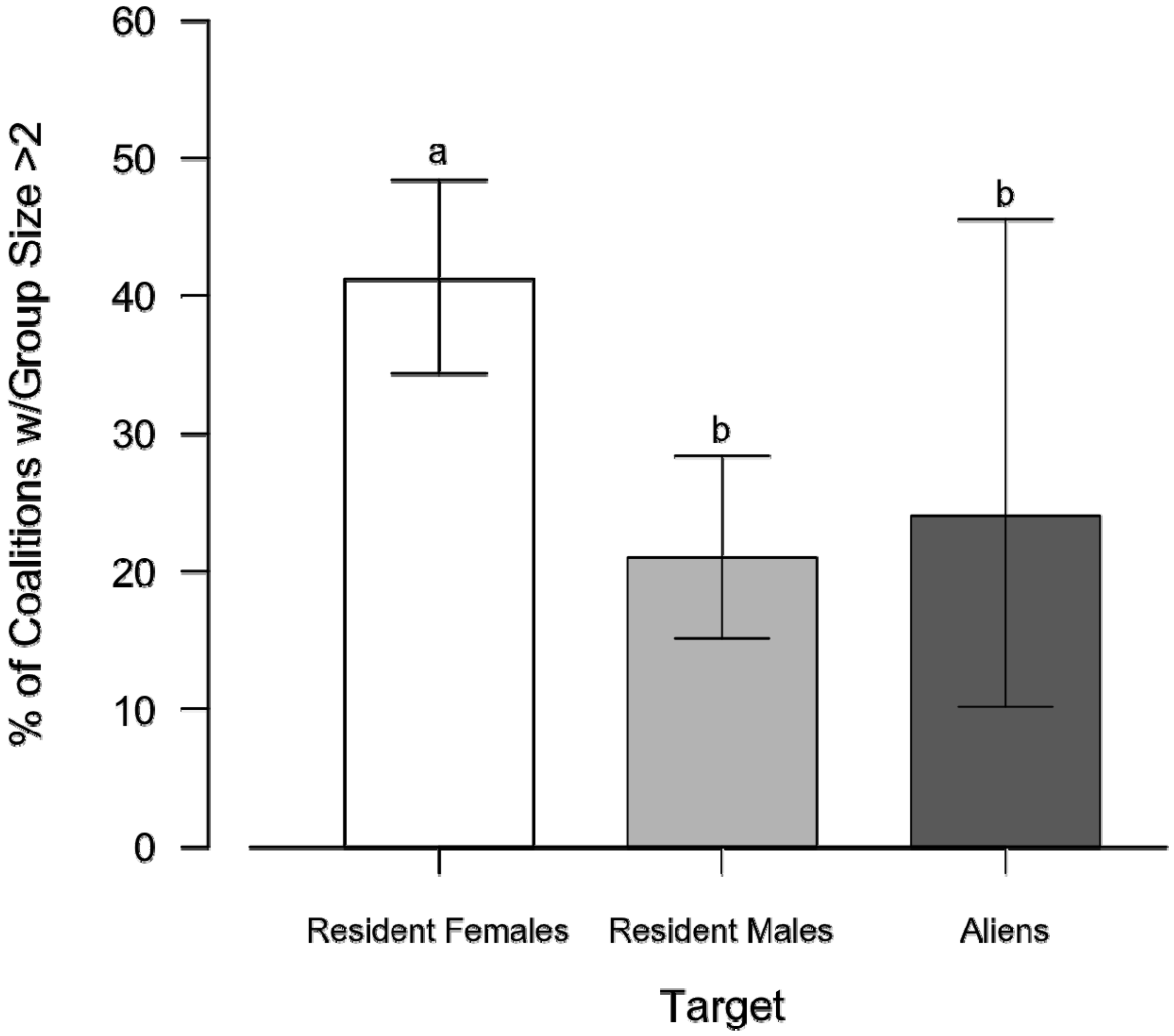

Figure 4.3. Boxplots of association indices among pairs of immigrant spotted hyenas (*Crocuta crocuta*) during various six-month periods during their relationship. Each number on the x-axis indicates the end of the period; e.g., year=1 represents the period from 0.5-1 year, year=1.5 represents the period from 1-1.5 years, etc. Horizontal lines indicate median association indices, box edges show 1st and 3rd IQR, and whiskers represent closest data points within (1.5 x IQR) of box edges. Numbers above bars indicate the number of pairs analyzed in that 6-month period. Outliers are not shown but were included in all analyses.

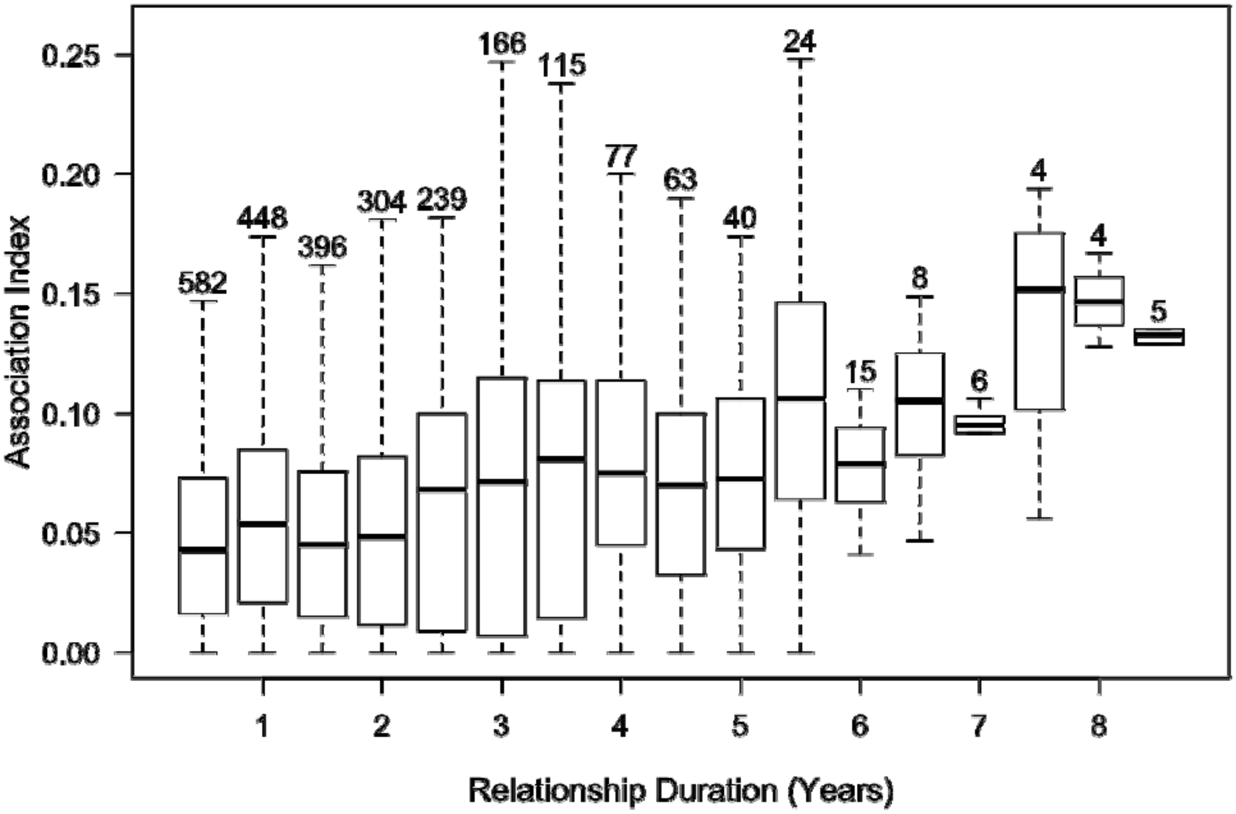

Figure 4.4. The probability that an immigrant male spotted hyena (*Crocuta crocuta*) participated in a coalition with other immigrants as a function of his maximum relatedness to the other member(s) of the coalition (referred to as R_M in the text). Relatedness was estimated using Queller-Goodnight *R* values. The solid black line represents the posterior mean estimate of the probability, and the dotted black lines represent the 95% credible intervals. The points represent the raw data indicating a potential cooperator's maximum relatedness to the actual cooperator(s) and his subsequent decision to participate in the coalition (=1) or not participate (=0). These results correspond with those from model_{KS} (Table 4.1).

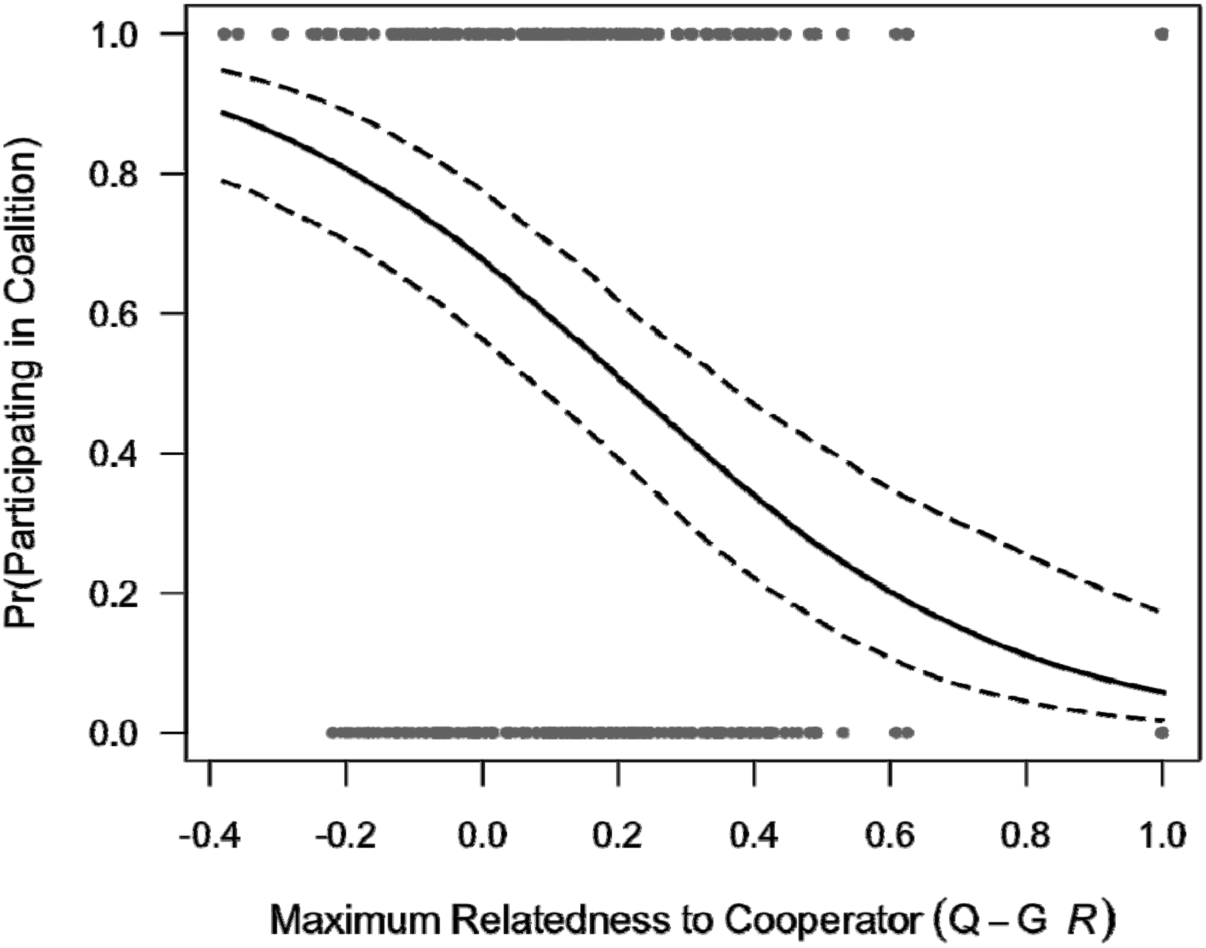

Figure 4.5. The probability that an immigrant male spotted hyena (*Crocuta crocuta*) participated in a coalition with other immigrants as a function of his social rank relative to the target of the coalition (the left-hand point indicates the potential cooperator is higher-ranking than the target). Posterior mean estimates of the probabilities are presented with error bars representing the 95% credible intervals. Different letters indicate that the corresponding posterior means were significantly different from one another. These results correspond with those from model$_{SQ}$ (Table 4.1).

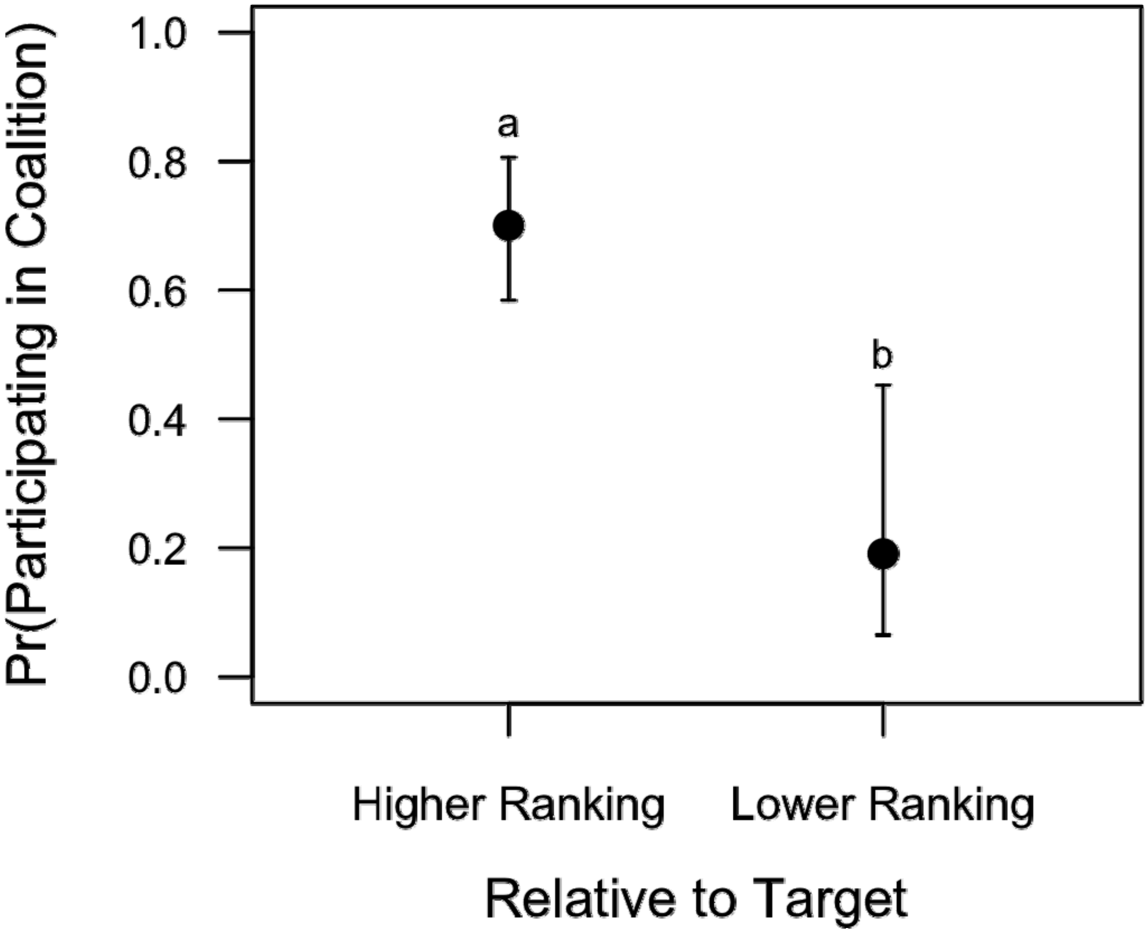

Figure 4.6. The number of cubs an immigrant male spotted hyena (*Crocuta crocuta*) sired during his total tenure in the clan as a function of his lifetime participation in coalitions with other immigrants against adult females (i.e., baiting episodes). The solid black line represents the posterior mean estimate of the number of cubs a male is expected to sire at a given lifetime participation in baiting episodes, and the dotted black lines represent the 95% credible intervals. The gray points represent the raw data used to generate the model. The inset shows a close-up version of the posterior mean estimate and 95% credible intervals, without the inclusion of the raw data, for finer-scale interpretations. These results correspond with those from model$_{RS}$ (Table 4.1).

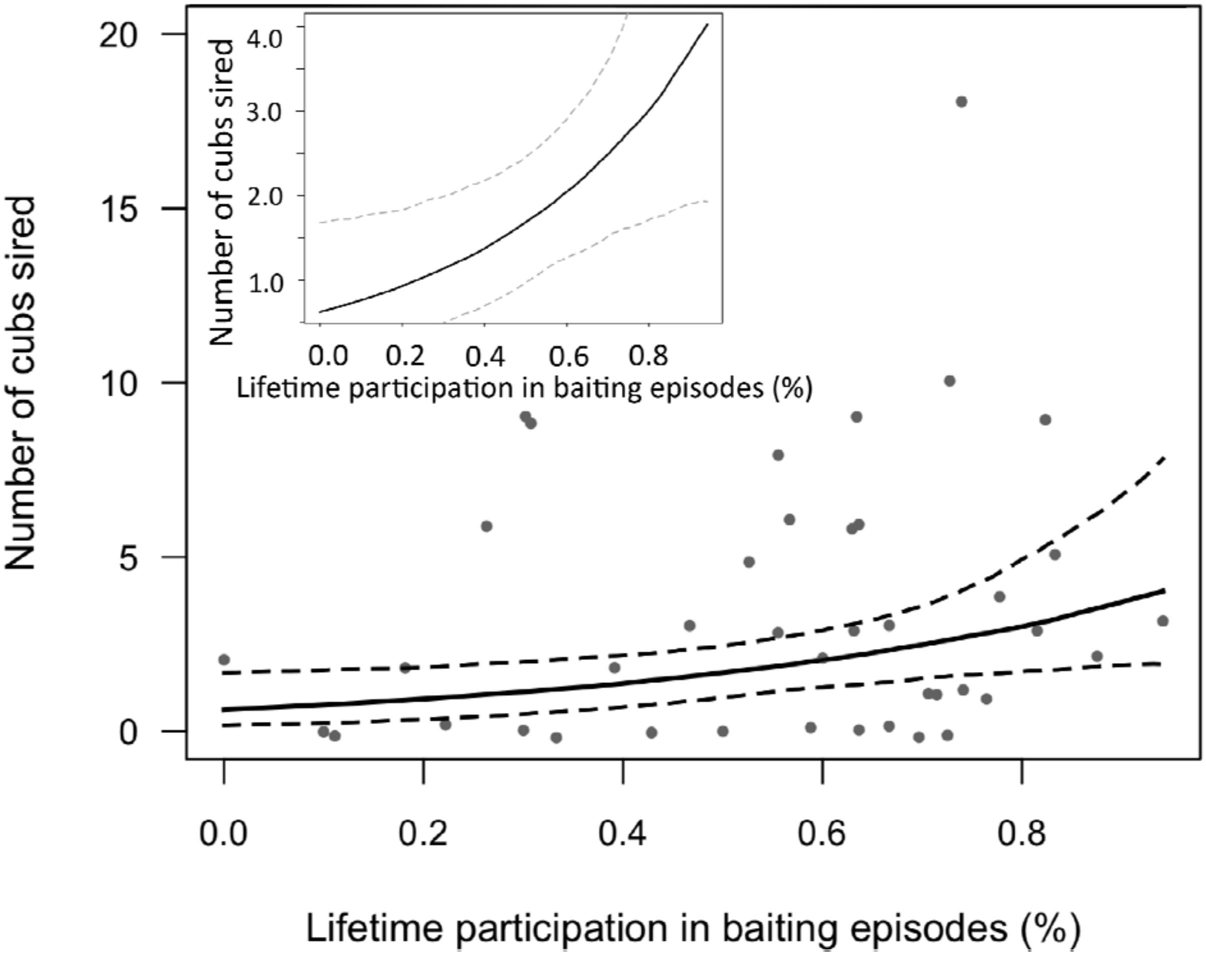

Table 4.1. Estimates of Bayesian mixed models using MCMCglmm. Posterior mean estimates of the fixed effect coefficients are reported with the 95% credible intervals (CI) and *p*MCMC values. Model_{AI} uses the Gaussian distribution, so the absolute values of the reported coefficients can be interpreteted without back transformations. Model_{AC}, model_{KS}, model_{SQ}, model_{FA}, and model_{RI} all use the logit-link function, so the reported coefficients are logit-transformed; in order to interpret the absolute values of the coefficients, one must take the inverse logit of the reported values. In model_{KS} and model_{SQ}, positive coefficients indicate a higher likelihood that a potential cooperator would indeed participate in a coalition relative to the intercept. In model_{FA} and model_{RI}, positive coefficients indicate a higher likelihood that an individual would retreat relative to the intercept. In model_{RS}, which uses the log-link function, the reported posterior mean estimates are the log-transformed values of the coefficients and represent the number of cubs a male would be expected to sire over his lifetime in the clan relative to the intercept. Therefore, in order to interpret the absolute values of the coefficients, one must take the inverse of the natural log of the reported value. Significant effects are presented in bold. Random effects are presented as the percent of the total variance they explained. All continuous covariates were standardized prior to their inclusion in the model.

Table 4.1 (cont’d)

Model	Sample size	Parameter	Posterior mean (95% CI)	*p*MCMC
model$_{AI}$	74 hyenas,	Intercept	-3.079 (-3.143, -3.011)	-
	624 pairs	**Relationship period**	**0.105 (0.086, 0.125)**	**<0.001**
		Random effects:	Percent of Variance:	
		Hyena ID	0.052	-
		Pair ID	70.912	-
model$_{AC}$	59 hyenas,	Intercept	-2.416 (-2.665, -2.179)	-
	431 pairs	**Lifetime association index**	**0.713 (0.501, 0.919)**	**0.002**
		Random effects:	Percent of Variance:	
		Hyena ID	2.929	-
		Pair ID	62.527	-
model$_{KS}$	52 potential cooperators,	Intercept (immigrant target)	0.214 (-0.294, 0.620)	-
	71 targets,	Target: female	0.308 (-0.152, 0.851)	0.242
	181 sessions,	Intxn between Q-G R_T and target status:		
	317 coalitions	Q-G R_T, immigrant target	-0.014 (-0.267, 0.201)	0.924
		Q-G R_T, female target	-0.172 (-0.481 0.176)	0.320
		Intxn between Q-G R_M and target status		
		Q-G R_M, immigrant target	**-0.761 (-1.057, -0.507)**	**<0.001**
		Q-G R_M, female target	0.208 (-0.095, 0.569)	0.222
		Random effects:	Percent of Variance:	
		Potential cooperator ID	25.522	-
		Target ID	13.631	-
		Session	12.028	-
		Coalition ID	6.398	-
model$_{SQ}$	57 potential cooperators,	Intercept (Potential cooperator is higher ranking than target)	0.848 (0.340, 1.423)	-
	47 targets,	**Potential cooperator is lower ranking than target**	**-2.293 (-2.999, -1.615)**	**<0.001**
	102 sessions,	Random effects:	Percent of Variance:	
	145 coalitions	Potential cooperator ID	16.653	-
		Target ID	21.734	-
		Session	11.645	-
		Coalition ID	6.090	-

Table 4.1 (cont'd)

model$_{FA}$	45 aggressors, 65 targets	Intercept (dyadic aggressive acts)	0.100 (-0.362, 0.543)	-
		Coalitionary aggressive acts	**1.963 (0.798, 3.124)**	**<0.001**
		Random effects:	Percent of Variance:	
		Aggressor ID	15.094	-
		Target ID	19.128	-
model$_{RI}$	46 aggressors	Intercept (dyadic aggressive acts, target ultimately joins clan)	-1.713 (-3.067, -0.4827)	-
		Coalitionary aggressive acts, target ultimately joins clan	**2.1544 (0.510, 3.786)**	**0.004**
		Dyadic aggressive acts, target does not ultimately join clan	0.793 (-0.842, 2.257)	0.306
		Coalitionary aggressive acts, target does not ultimately join clan	-1.643 (-3.637, 0.377)	0.114
		Random effects:	Percent of Variance:	
		Aggressor ID	30.241	-
model$_{RS}$	41 hyenas	Intercept	0.602 (0.146, 0.987)	-
		Tenure	**0.906 (0.489, 1.401)**	**<0.001**
		% Participation in baiting episodes	**0.465 (0.065, 0.879)**	**0.018**
		Tenure*% Participation in baiting episodes	0.045 (-0.362, 0.462)	0.810

Table 4.2. Results of partial rowwise matrix correlation tests for reciprocal coalitionary support among immigrant male spotted hyenas (*Crocuta crocuta*). Bolded records indicate statistically significant *TauKr* correlation coefficients.

Time Period	Duration (years)	Number of Dyads	*TauKr*	*p*-value
1988 – 2009	**22**	**211**	**0.067**	**0.019**
1990 – 1994	5	61	0.127	0.071
1996 – 1999	4	118	0.042	0.171
2001 – 2006	**6**	**28**	**-0.162**	**0.020**

APPENDIX

APPENDIX C

We arranged all MCMCglmm (Hadfield 2010) specifications to produce 1000 posterior samples, which constituted the posterior distribution for each model and were used in the calculation of all posterior estimates. We ran each model until the autocorrelation for successive iterations was <0.2 for both fixed effects and variance components (Plummer et al. 2006; Hadfield 2010), and until the model reached proper convergence, which we assessed visually using trace plots and computationally using Geweke's convergence diagnostic (Plummer et al. 2006). The total number of iterations therefore varied among models; see Table C1 for complete model specifications. We ran three parallel (identical but independent) MCMC chains for each model and checked convergence among the three chains using potential scale reduction factors (Gelman & Rubin 1992). The posterior mean estimates and posterior distributions were taken from the first of these three chains. We used variance inflation factors and condition indices to assess the multicollinearity of our models (Heiberger 2009; Hendrickx 2010). All statistical analyses were carried out in R v. 2.13.0 (R Development Core Team 2011).

Bayesian modeling techniques, such as those used here, require priors to be specified for each model. Priors for fixed effects have two parameters, the mean and the variance, and for all models we used MCMCglmm's default prior, which is a normal prior centered around zero and with a large variance (Hadfield 2010). The priors used for random effects also have two parameters: the variance (V) and the degree of belief in that variance (nu). The final prior pertains to the residual variance; this prior specification is often identical to the prior specified for the random effects, but in certain distributions the residual variance cannot be estimated and is instead fixed at some value. For all models, we tried a variety of priors and found the model results to be highly robust; our final prior specifications are reported in Table C1.

Table C1. Summary of the MCMCglmm specifications of each model (Hadfield 2010). "Distribution" is labeled according to MCMCglmm naming conventions. "Thin" refers to the thinning interval used.

Model	Distribution	# Iterations	Burn-In	Thin	Prior for R.E.[a]	Prior for R.V.[b]
model_{AI}	Gaussian	200,000	50,000	150	V=1, nu=0.002	V=1, nu=0.002
model_{AC}	binomial	600,000	150,000	450	V=1, nu=1	V=1, nu=0.002, fix=1
model_{KS}	categorical	300,000	100,000	200	V=1, nu=1	V=1, fix=1
model_{SQ}	categorical	300,000	100,000	200	V=1, nu=1	V=1, fix=1
model_{FA}	categorical	3,000,000	1,000,000	2000	V=1, nu=1	V=1, fix=1
model_{RI}	categorical	3,000,000	1,000,000	2000	V=1, nu=1	V=1, fix=1
model_{RS}	Poisson	500,000	200,000	300	-	V=1, nu=0.002

[a] Random Effects.

[b] Residual Variance.

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