



## Faecal androgen concentrations in adult male spotted hyaenas, *Crocuta crocuta*, reflect interactions with socially dominant females

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Androgens influence many physiological processes in male vertebrates and have been linked to variation in aggression and reproductive behaviour in many species. This study examined relations between social behaviour and androgens excreted by adult male spotted hyaenas. In this species, males rarely direct reproductive aggression towards other males, but they actively court females that are socially dominant to them. We tested whether intersexual interactions were associated with elevated androgens in adult males. We combined behavioural data collected over 7 years from one large clan in Kenya with analysis of faecal androgens (fA) to determine which of the following were the best predictors of fA concentrations in males: social rank, tenure in the clan, courtship, association with females in particular reproductive states, reproductive aggression among males or nonreproductive aggression among males. We found no systematic influence of age or social status on fA in either adult natal or immigrant males. Concentrations of fA were greater in immigrants when they courted females than at other times, and greater fA concentrations were also associated with male–male aggression involving defence of a female but not with male–male aggression occurring in other contexts. fA concentrations in immigrants were also positively correlated with degree of association with females near the time of conception, but not with lactating females. These findings suggest that androgen concentrations in immigrant male hyaenas are more related to interactions with attractive females than to competitive male interactions. The most important challenge to adult males may be interacting with attractive females to which males are socially subordinate.

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Linking physiology with reproductive behaviour is critical to our understanding of the proximate mechanisms involved in the regulation of reproductive success, and socioendocrine studies can shed light on sources of individual variation in reproductive success (Bercovitch 1999; Bercovitch & Ziegler 2002). In male vertebrates, androgens, particularly testosterone (T), influence a variety of morphological and behavioural processes related to reproduction and social behaviour. Testosterone is essential

for the formation of secondary sexual characteristics (Wickings & Dixson 1992; Dixson 1998; Maggioncalda et al. 1999; Gonzalez et al. 2001), is crucial for the initiation and maintenance of sperm production and sexual behaviour (Wickings et al. 1986; Baum 2002) and has been linked to the expression of aggressive behaviour and social status (Harding 1981; Bouissou 1983; Albert et al. 1990; Whitten 2000). Androgen concentrations can be quite variable between individuals in a population, and this variation may be associated with individual differences in behaviour, physiology and reproductive success. The relation between androgens and behaviour is often modulated by social factors. For example, concentrations of T in primates often do not correlate with dominance status or aggressive behaviour under stable social conditions (Sapolsky 1983; Cavigelli & Pereira 2000), but is positively correlated with situations characterized by unstable hierarchies or other social challenges (Sapolsky 1983;

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Steklis et al. 1986; Bernstein et al. 1992; Cavigelli & Pereira 2000; Brockman et al. 2001).

Although originally developed for use in seasonally breeding avian species, the 'challenge hypothesis' (Wingfield et al. 1990, 2000) has recently been used as a theoretical framework to help predict patterns in the relationship between T and aggression associated with mating in various species of mammals (dwarf mongoose, *Helogale parvula*: Creel et al. 1993; wild dog, *Lycaon pictus*: Creel et al. 1997; ringtailed lemur, *Lemur catta*: Cavigelli & Pereira 2000; Verreaux's sifaka, *Propithecus verreauxi*: Brockman et al. 2001; tufted capuchin monkey, *Cebus apella nigritus*: Lynch et al. 2002; redfronted lemur, *Eulemur fulvus rufus*: Ostner et al. 2002; spotted hyaena, *Crocuta crocuta*: Goymann et al. 2003; Arctic ground squirrel, *Spermophilus parryii*: Buck & Barnes 2003; chimpanzee, *Pan troglodytes*: Muller & Wrangham 2004). As originally formulated, the main premise of the challenge hypothesis is that the relation between T and aggression during the breeding season in males of a given species can be predicted by the level of aggressive male–male competition and extent of paternal care characteristic of that species. In species where males are intensively involved in parental care and engage in relatively low rates of male–male aggressive competition (often monogamous species), the hypothesis predicts that males should maintain a breeding baseline level of T and respond to occasional aggressive challenges with a relatively large increase in T to facilitate aggressive competition. In contrast, in species showing no paternal care and higher rates of male–male aggression (often polygynous species), the hypothesis predicts that males should maintain relatively high concentrations of T during the entire breeding season to facilitate a heightened state of aggression. Therefore, the physiological response of a male to challenge in this situation should be relatively small, because T is already at or near its effective maximum (Wingfield et al. 1990, 2000; Creel et al. 1993).

Although the majority of studies testing the challenge hypothesis have focused on the relation between androgens and reproductive aggression among same-sexed individuals, the original hypothesis also suggests that physiologically influential social challenges may occur during interactions with potential mates as well as during interactions with rival males (Wingfield et al. 1990, 2000). Male white-crowned sparrows, *Zonotrichia leucophrys*, that are caged with oestradiol-implanted females have higher concentrations of T than do males housed with nonreceptive females (Moore 1983). In European starlings, *Sturnus vulgaris*, testosterone concentrations are positively influenced by female presentation (Pinxten et al. 2003). Most recently, Moore et al. (2004) found that the challenge hypothesis was not supported in an equatorial population of rufous-collared sparrows, *Zonotrichia capensis*, and suggested that male–female interactions may be responsible for patterns of T observed in this species. Plasma T concentrations also rise in many male mammals after mating or exposure to a novel receptive female (Katongole et al. 1971; Jainudeen et al. 1972; Rose et al. 1972; Purvis & Haynes 1974; Sanford et al. 1974; Macrides et al. 1975). These studies suggest that, when investigating sources of variation in androgen concentrations in males, it may be

important to consider the influence of intersexual interactions in addition to the influence of aggressive competition with rival males.

We evaluated the influence of intersexual and intra-sexual interactions on male androgen excretion in a gregarious carnivore, the spotted hyaena. These hyaenas live in dynamic fission–fusion groups of up to 80 individuals, called clans. Each clan contains one to several matrilineal lines of adult females and their offspring, as well a number of immigrant males. Clans are structured by hierarchical rank relationships (Kruuk 1972; Tilson & Hamilton 1984; Frank 1986; Holekamp & Smale 1990), and all adult females are socially dominant to all adult males not born in the clan (Kruuk 1972; Smale et al. 1993). Adult males are less aggressive than adult females (Kruuk 1972; Frank 1986; Mills 1990; Szykman et al. 2003), and the social rank of individuals within the male hierarchy is acquired by queuing, rather than by aggression (East & Hofer 2001; Engh et al. 2002). Males are seldom observed to engage in aggressive interactions, and what little aggression does occur between males is typically mild. As a result of female dominance and morphological masculinization of the external genitalia, females appear to have unusually tight control over reproduction (Kruuk 1972; East et al. 2003), and the awkward courtship behaviour of the male hyaena reflects his extreme motivational conflict with regard to approaching females (Holekamp & Smale 1998; Szykman 2001). Molecular genetic data have revealed that high-ranking males do not monopolize reproduction, that both males and females mate with multiple partners, and that at least 20% of twin litters are of mixed paternity (Engh et al. 2002; East et al. 2003). Males show no paternal care (Kruuk 1972), although they can recognize their own offspring (Van Horn et al. 2004).

Female hyaenas are philopatric, but males disperse sometime after reaching reproductive maturity at approximately 2 years of age. Males may remain in their natal clans for up to 52 months after puberty (Smale et al. 1997; East & Hofer 2001; Van Horn et al. 2003), so there are two classes of adult males in every clan: natal males born in the clan and immigrant males born elsewhere. Although natal males are socially dominant to all immigrant males, immigrants engage in higher rates of sexual and courtship behaviour, have higher average concentrations of plasma testosterone (Holekamp & Smale 1998; Holekamp & Sisk 2003; Dloniak et al. 2004), and sire 30 times more offspring than do natal males (Engh et al. 2002).

Goymann et al. (2003) described a relation in spotted hyaenas between elevated plasma T and the occurrence of male defence of one or more females. However, low-intensity male–male reproductive aggression in the form of female defence is not common among male hyaenas, and we therefore suggest that the relation between mate defence and androgens may be confounded by the presence of a female and/or by courtship interactions between the sexes. We therefore tested the hypothesis that intersexual interactions constitute a significant social challenge for male hyaenas, and that these interactions correlate with androgen concentrations. We recently developed an assay to quantify faecal androgen (fA) concentrations in this species noninvasively (Dloniak et al. 2004), and

we used that assay with samples collected from adult male spotted hyaenas to evaluate the influence of the following variables on fA concentrations: social status, tenure in the clan, reproductive aggression between males, male–male aggression not associated with the defence of a female, courtship and degree of association with females in particular reproductive states. If male–male competition represents a significant challenge for male hyaenas, fA concentrations should vary with the occurrence of male–male aggression. On the other hand, if interactions with behaviourally dominant yet attractive females constitute significant challenges for males, we expected fA concentrations in males to vary with social measures related to interactions with females. We also predicted that relationships between androgens and behaviour would differ between adult natal males and immigrant males, based on documented differences in behaviour and physiology between these two groups.

## METHODS

### Study Site and Animals

The study was conducted in the Talek area of the Masai Mara National Reserve, an area of open rolling grassland in southwest Kenya. The subject population was one large, stable spotted hyaena clan, inhabiting a home range of about 65 km<sup>2</sup> (Boydston et al. 2001). The Talek clan has been intensively studied since 1988, and the boundaries of the clan's home range have been stable at least since 1979 (Frank 1986). All hyaenas in the clan were known individually by their unique spot patterns, and sex was determined by the dimorphic glans morphology of the erect phallus (Frank et al. 1990). Data were gathered between July 1995 and July 2002; observers monitored the Talek hyaenas 20–31 days per month for approximately 6 h per day. During this study period, the number of adult immigrant males, adult natal males and adult females in the clan at any one time ranged from 11–21, 1–9 and 21–31, respectively.

Adult natal males were those that had been born in the Talek clan, and were older than 24 months (the approximate age of puberty) but had not yet dispersed. Ages of adult natal males were known  $\pm 7$  days, using methods described by Holekamp & Smale (1998). Ages of the adult natal males ranged from 26 to 68 months. Adult immigrant males were those that had been born outside Talek and then immigrated into the Talek clan at dispersal. These males originated in clans other than our study population, so their exact ages were unknown. However, we could reliably estimate ( $\pm 6$  months) the ages of immigrant males based on tooth-wear measures obtained during routine immobilizations (Van Horn et al. 2003), and these ages ranged from 30 to 150 months. We also recorded the date of first appearance in the Talek home range of each immigrant male, thus allowing us to calculate his tenure in the clan at the time of each sampling, based on time elapsed since his initial appearance in the clan.

We monitored the extent to which adult males associated with adult females that were either attractive (close to conception) or lactating (relatively unattractive). Spotted hyaenas in East Africa breed year-round (Holekamp et al. 1999), so at any time there may be pregnant, lactating and cycling females present in a clan. Lactating females were within the third or fourth month of lactation, based on direct observation of nursing bouts and known birth dates of cubs. Attractive females were within one month of a known conception. We calculated the conception date for each litter born to each female by subtracting 110 days, the length of the gestation period in *Crocuta*, from the birth date of that litter (Schneider 1926). Birth dates could be reliably estimated to  $\pm 7$  days (Smale et al. 1993).

### Behavioural Observations

We conducted daily behavioural observations from vehicles between 0530 and 0930 hours and between 1630 and 2000 hours. We located hyaenas while driving daily circuits around the Talek home range, visiting high points in the area and scanning with binoculars, and by radio-tracking collared individuals. We initiated an observation session when we first drove up to one or more hyaenas separated from others by at least 200 m, and the session ended when all hyaenas moved out of sight or when we drove to a new location. Data documenting agonistic behaviour during observation sessions were collected using critical incident sampling (Altmann 1974) of all aggression and appeasement behaviours observed in all social contexts. All courtship behaviours were also recorded as critical incidents between May 1997 and August 1999, and between May 2000 and May 2002. Social ranks were assigned based on a matrix of outcomes of dyadic agonistic interactions (Martin & Bateson 1988), as described previously (Smale et al. 1993). Adult females, together with their offspring, and adult immigrant males were ranked in separate dominance hierarchies. The number of immigrant males present during the study varied over time, so we calculated a standardized social rank for each immigrant at each time of sampling by assigning the highest-ranking male a rank of +1 and the lowest-ranking male the rank of –1, with all other males spread evenly between these two individuals (East & Hofer 2001; Goymann et al. 2003).

### Agonistic behaviour

Appeasement behaviours used to determine social ranks included headbob, carpal crawl, giggle, squeal, back-off and submissive posture (tail between the legs, head down, body lowered and bent; Kruuk 1972; Holekamp & Smale 1998). Aggression included lunge, snap, bite, chase, displace, push, stand over and intention movement to bite (Kruuk 1972; Holekamp & Smale 1998). We used social context to distinguish between reproductive and nonreproductive aggression between males. For a male to show reproductive aggression, he had to be within 10 m of an adult female and show aggression (including at least a low-level threat, such as displace) towards another adult male who approached to within 10 m of that female. If

the context of the aggression was not related to a female (such as during feeding with other males), we considered it to be nonreproductive aggression. We discarded aggressive interactions for which context was ambiguous.

### Courtship behaviour

Courtship behaviour included extended following, approach/avoid display, present, bowing display, paw the ground and mount (definitions in Holekamp & Smale 1998; Szykman 2001). Adult male hyaenas show these behaviours only towards females as indicators of male sexual interest (Holekamp & Smale 1998; Szykman 2001). Although these behaviours were often accompanied by appeasement gestures, courtship behaviours alone could not be used to determine social rank, because males never directed them at other males. We did not include either male aggression towards females (baiting behaviour: Kruuk 1972; Szykman et al. 2003) or copulations in our analyses because of small sample sizes for these rarely observed events.

### Association index

We assessed whether the degree of association between a male and a particular female influenced androgen concentrations in the male's faeces. The highly dynamic, fission–fusion nature of spotted hyaena society means that the best measure of association between two individuals is an association index (AI). We therefore calculated AIs around a male's faecal sample date between that male and all members of each of the two classes of adult female, attractive and lactating, that were present in the clan at that time. We used the twice-weight index of association (Cairns & Schwager 1987):

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

where  $(A_{\text{without } B})$  represents the number of observations in which male A was observed but female B was not present,  $(B_{\text{without } A})$  represents the number of observations in which female B was present but male A was not, and  $(A+B)_{\text{together}}$  represents the number of sessions in which both male A and female B were present (Szykman et al. 2001). For 69 faecal samples from 22 immigrant males, we separately calculated male AIs with both lactating and attractive females present in the clan for the period encompassing three weeks before and one week after the date of faecal sample collection.

### Faecal Sample Collection, Processing and Androgen Assay

During the 7-year study period, faecal samples were collected from 16 adult natal males and 26 adult immigrant males, yielding 25 and 159 samples, respectively. A faecal sample was collected whenever a hyaena was directly observed to defecate during an observation session. Samples were first collected into plastic bags at the site of defecation, then mixed and transferred to 3-ml cryovials. Samples were stored in liquid nitrogen until transported on dry ice to the United States, where they

were stored at  $-20^{\circ}\text{C}$  or colder until processing. Samples were then extracted and assayed for androgens as described by Dloniak et al. (2004). In brief, steroids in 0.2-g lyophilized faeces were extracted with 100% ethanol. Faecal extracts were reconstituted and diluted 1:50 in PBS and measured for immunoreactive androgens by enzymeimmunoassay (EIA). The assay used a testosterone antibody (R156/7) and a testosterone conjugate (horseradish peroxidase) provided by Bill Lasley and Coralie Monroe of the University of California at Davis. The mean coefficient of variation between assays ( $N = 10$ ) was 8.5%, based on high and low pools in each assay. The intra-assay coefficient of variation for high and low pools run within each assay was 7.3%. Using over 600 faecal samples, Dloniak et al. (2004) demonstrated that neither time of day at which the sample was collected nor the duration that the sample was kept in frozen storage before extraction and assay systematically influenced fA concentrations in spotted hyaenas, so we did not include these variables in our analyses.

### Statistical Analyses

Adult natal and immigrant male hyaenas behave very differently, have different mean plasma T and fA concentrations, and respond differently to challenge with gonadotrophin releasing hormone (Holekamp & Smale 1998; Holekamp & Sisk 2003; Dloniak et al. 2004), and we collected many more faecal samples from immigrants than from natal males. Therefore, we analysed the relationships between fA and behaviour in each of these two groups of males separately. We first evaluated whether rates of courtship were higher than rates of reproductive aggression in six adult natal males and 12 adult immigrant males present in the Talek clan for the entire period between May 2000 and May 2002, using Wilcoxon matched-pairs analyses. In adult natal males, we then investigated relations among fA, age and social rank at the time of sampling using multiple regression. Some data points were nonindependent (25 samples from 16 males, including 2 males with 3 samples each and 5 males with 2 samples each), so we included individual hyaena identity (ID) as a random effect variable in a generalized linear mixed model with backward selection. We next analysed whether natal males showing nonreproductive aggression, reproductive aggression or courtship behaviour during the 3 days before and on the day of a faecal sample had greater fA concentrations than did natal males not showing these behaviours at the time of sampling. We chose this time frame because circulating androgens are represented in hyaena faeces 1–4 days later (Dloniak et al. 2004). Three separate analyses were done using Mann–Whitney  $U$  tests with the samples categorized by the presence/absence of the behaviours. The same 25 samples were used in these three analyses, so we corrected for the experimental error rate by applying a sequential Bonferroni adjustment in significance testing (Rice 1989). Because of the small number of samples obtained from natal males, we did no further analysis of this data set.

In immigrant males, we first used multiple regression to investigate relations among fA, estimated age, standardized social rank and tenure in the clan. The majority of adult immigrant males were sampled at least three times throughout the study, and the number of samples per individual varied greatly (range 1–17, mean = 6). Because of this unbalanced data set, we included hyaena identity as a random effect variable in the initial regression. Identity had no significant effect, so we repeated the analysis without including identity. We next used subsets of the available samples to analyse the relations between fA and behaviour in immigrants. We first determined which faecal samples were associated with the observation of the male engaging in the behaviours of interest. Then we chose a paired sample from the same male that was the nearest in time, but where he did not show the behaviour. We used Wilcoxon matched-pairs tests to determine whether fA concentrations in males showing nonreproductive aggression, reproductive aggression or courtship behaviour were different from concentrations in the same males when they were not showing these behaviours. No sample was used in more than one analysis, and a male was represented by only one paired sample within an analysis. We again used multiple regression to analyse the relation between immigrant male fA and AIs with lactating and attractive females.

All statistical analyses followed Zar (1996) and were done using Statistica 8.0 (Tulsa, Oklahoma, U.S.A.). Statistical significance was set at  $\alpha = 0.05$  and nonparametric tests were used when necessary. Results are presented as means  $\pm$  standard errors. The statistical power of important nonsignificant tests was investigated using Gpower (Faul & Erdfelder 1992).

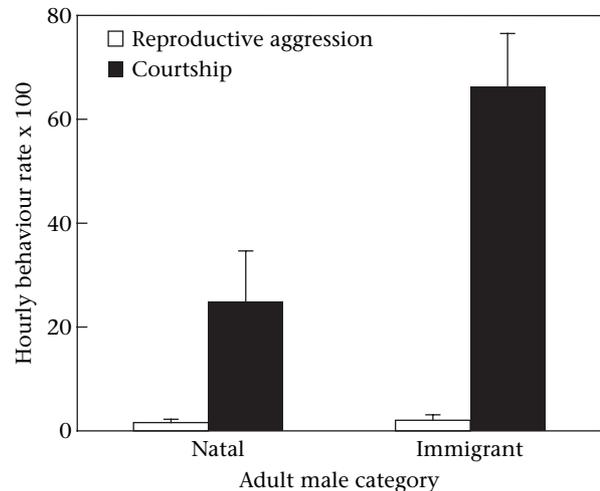
## RESULTS

### Rates of Reproductive Aggression and Courtship

A comparison of courtship and reproductive aggression in six adult natal males that were present in the Talek clan throughout a 2-year period showed that the rate at which these males directed courtship behaviour towards females was significantly higher than the rate at which they directed reproductive aggression towards other males (Wilcoxon matched-pairs test:  $T = 2.397$ ,  $P = 0.0418$ ; Fig. 1). In a corresponding analysis of 12 immigrant males present in the Talek clan during the same interval, immigrant males also showed much higher rates of courtship towards females than they did reproductive aggression towards other males ( $T = 6.357$ ,  $P = 0.0022$ ; Fig. 1). Reproductive aggression among adult males generally consisted of low-level threats, and these were always directed towards subordinate males.

#### Adult Natal Males

We found no correlation between social rank, age and fA concentrations in adult natal males, controlling for hyaena identity as a random effect in a mixed model multiple



**Figure 1.** Paired rates of reproductive (male–male) aggression and courtship observed in adult male Talek hyaenas, May 2000–May 2002.  $N = 6$  natal males and 12 immigrants. Rates of behaviour were compared within each category.

regression (whole model:  $R = 0.413$ ,  $F_{3,21} = 1.4389$ ,  $P = 0.26$ ). This may have resulted from low power to detect relations because of small sample size. The power to detect a medium effect size was 0.35, and the power to detect a large effect was 0.70. Because we found no effect of social rank or age in natal males, we did not control for these variables in subsequent analyses of behaviour. Concentrations of fA were similar between males showing nonreproductive aggression ( $\bar{X} \pm SE = 386.32 \pm 115.48$  ng/g) and males failing to show any nonreproductive aggression ( $315.27 \pm 88.70$  ng/g; Mann–Whitney  $U$  test:  $U = 67.00$ ,  $N_1 = 9$ ,  $N_2 = 16$ ,  $P = 0.7771$ ). We found no significant difference between fA concentrations in natal males showing courtship behaviour before a faecal sample ( $\bar{X} \pm SE = 308.12 \pm 87.62$  ng/g) and those in males showing no courtship behaviour ( $362.66 \pm 101.63$  ng/g;  $U = 73.00$ ,  $N_1 = 10$ ,  $N_2 = 15$ ,  $P = 0.9117$ ). There was a slight tendency for natal males showing reproductive aggression to have greater fA concentrations ( $\bar{X} \pm SE = 851.66 \pm 52.71$  ng/g) than natal males failing to show reproductive aggression ( $296.43 \pm 67.49$  ng/g), but this difference was not statistically significant when the Bonferroni adjustment was applied, possibly because of the low sample size ( $U = 3.00$ ,  $N_1 = 2$ ,  $N_2 = 23$ ,  $P = 0.0451$ ). Again, because of low sample size, the power of these analyses to detect even a large effect was low (0.58 for nonreproductive aggression, 0.60 for courtship, and 0.28 for reproductive aggression). The faecal samples of the two males that showed reproductive aggression had the highest concentrations of fA among all samples collected from adult natal males, and both of these males were also involved in courting females before the sample collection.

#### Immigrant Males

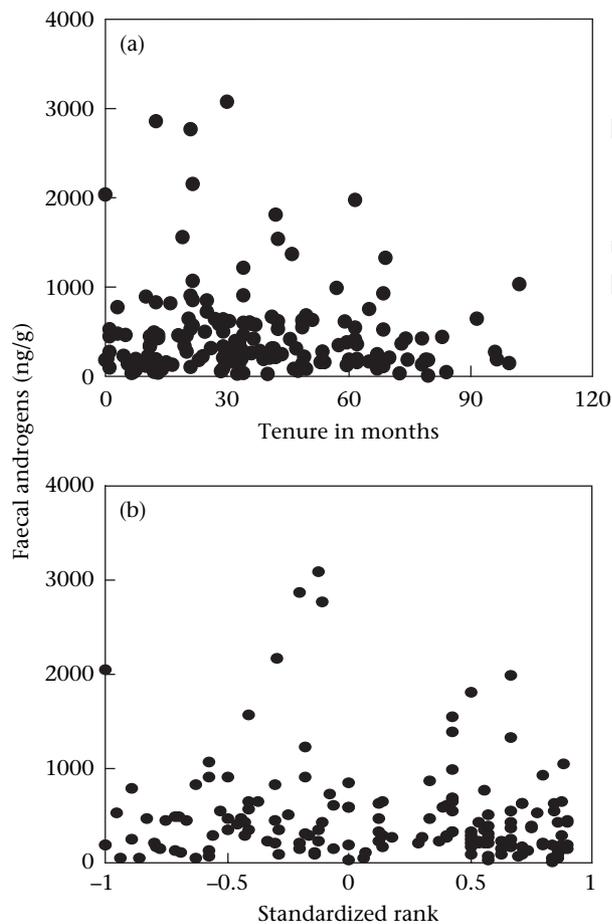
We first used multiple regression to investigate the influence of male social status, age or tenure on fA concentrations among immigrants. The overall model

here was significant (whole model:  $R = 0.25$ ,  $F_{4,134} = 4.78$ ,  $P = 0.01$ ), with tenure ( $P = 0.03$ , Fig. 2a) and standardized social rank ( $P = 0.03$ , Fig. 2b) retained, but hyaena identity and estimated age removed from the model by backwards selection. However, social rank and tenure were closely and positively correlated ( $R = 0.90$ ), neither variable explained significant variation in fA (social rank partial  $R = 0.03$ , tenure partial  $R = -0.13$ ), and the partial correlations with fA concentration were in opposing directions.

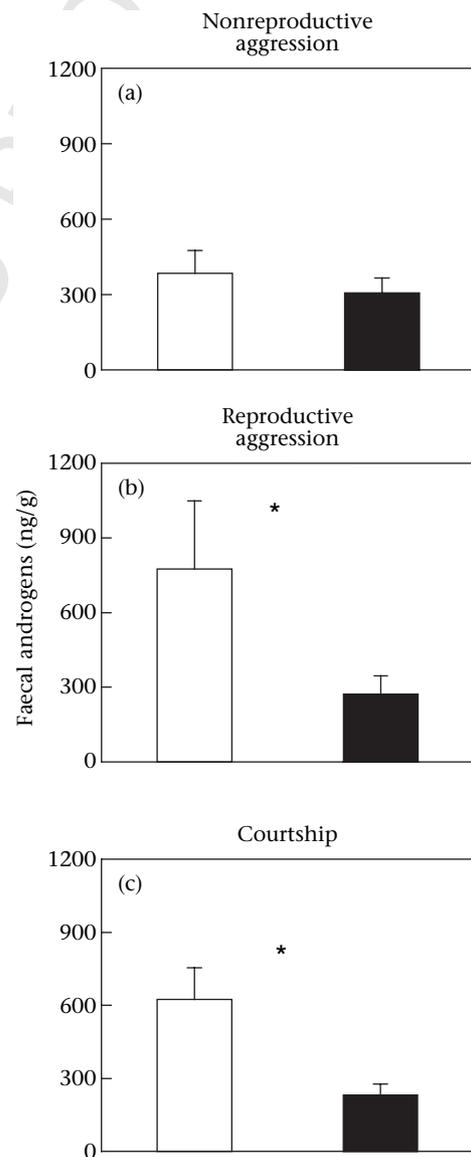
Samples from immigrant males were sufficiently large to permit use of paired analyses of the relations between androgens and specific behaviours. Although a male's fA concentrations did not vary significantly based on whether he showed nonreproductive aggression (Wilcoxon matched-pairs test:  $T = 27.00$ ,  $N = 13$ ,  $P = 0.1961$ ; Fig. 3a), fA concentrations were significantly higher in samples collected from immigrant males showing reproductive aggression than in those from the same males when they were not showing reproductive aggression ( $T = 1.00$ ,  $N = 6$ ,  $P = 0.0464$ ; Fig. 3b). fA concentrations were also greater in samples from immigrant males showing courtship behaviour than in samples from the same males when

they did not show courtship ( $T = 4.00$ ,  $N = 16$ ,  $P = 0.0009$ ; Fig. 3c). Faecal samples from only 6 of 26 males were associated with reproductive aggression. Of these six males, only five were also seen courting a female during the days before the sample (Fig. 3b). In the courtship analysis (Fig. 3c), we included only samples from males showing courtship behaviour without reproductive or nonreproductive aggression.

A potential confound in our behavioural analyses is that female presence is required for courtship and reproductive aggression but not for nonreproductive aggression. Therefore, we next tested whether the extent to which immigrant males associated with females influenced fA concentrations. The multiple regression model of AI was



**Figure 2.** Relations between faecal androgen concentrations in immigrant males and (a) their tenure in the Talek clan and (b) their standardized social rank (highest possible rank = 1) at the time of sampling.  $N = 159$  faecal samples from 26 males.



**Figure 3.** Differences in faecal androgen concentrations between samples from immigrant males when they did (□) and did not (■) show (a) nonreproductive aggression ( $N = 13$ ), (b) reproductive aggression ( $N = 6$ ) and (c) courtship ( $N = 16$ ). Males are paired between bars within each histogram. \* $P < 0.05$ .

significant (whole model:  $R = 0.565$ ,  $F_{2,66} = 15.506$ ,  $P < 0.00001$ ). The AI between males and lactating females was not correlated with the AI between these same males and attractive females ( $R = 0.035$ ). fA concentrations were positively correlated with AI between males and attractive females (partial  $R = 0.558$ ,  $P < 0.00001$ ; Fig. 4a), but not with AI between males and lactating females (partial  $R = 0.127$ ,  $P = 0.213$ ; Fig. 4b).

## DISCUSSION

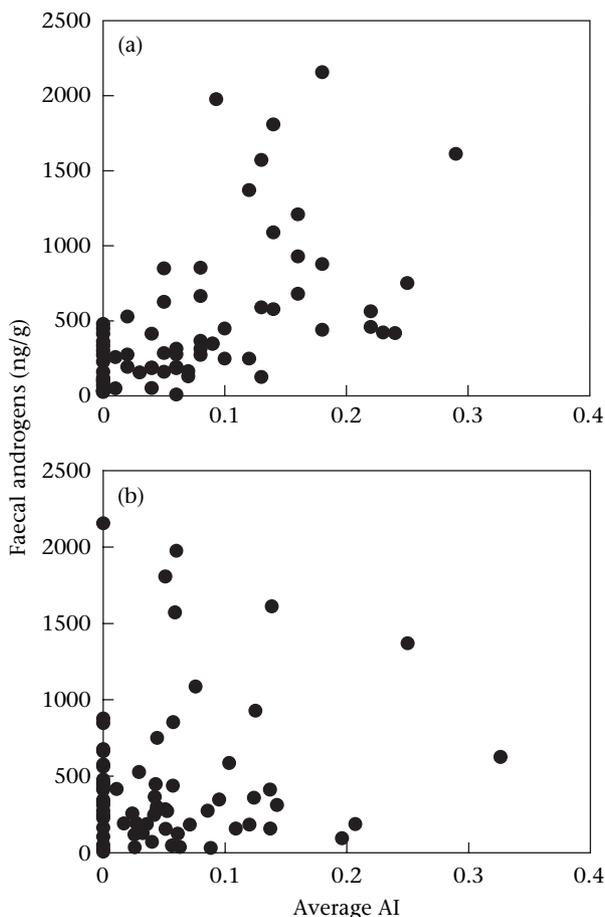
Although behaviour was not a useful predictor of fA concentrations in adult natal males, reproductive aggression and courtship were good predictors of elevated fA concentrations in adult immigrant males. Furthermore, degree of association with females near their times of conception was positively related to fA concentrations in immigrant males, but association with lactating females showed no such relation. These results suggest that close association with cycling females is related to reproductive physiology in the male spotted hyaena.

Our results indicate that age does not systematically influence fA concentrations in adult male spotted

hyaenas, regardless of dispersal status. Furthermore, neither adult natal nor immigrant males showed systematic status-dependent variation in fA concentrations within their social hierarchies. Although this lack of a simple relation between plasma T and social status has been shown in immigrant male hyaenas (Goymann et al. 2003; Holekamp & Sisk 2003), we found that fA concentrations show the same pattern as plasma T relative to social status, and that the lack of relation between androgens and social rank also holds true for adult natal males. Social ranks in natal males are based on the social ranks of their mothers (Holekamp & Smale 1990; Smale et al. 1993; Engh et al. 2000). Furthermore, immigrant males only gain status in their new clan by advancing in a queue as higher-ranking males die or emigrate (East & Hofer 2001; Engh et al. 2002). Thus, neither natal nor immigrant males gain social status through fighting ability or size. These results therefore resemble findings in other species of mammals that androgen concentrations are not closely related to male social status within a stable hierarchy (Eaton & Resko 1974; Gordon et al. 1976; Bernstein et al. 1983; Steklis et al. 1986; Alberts et al. 1992; Sapolsky 1993; Goymann et al. 2003).

The challenge hypothesis is often interpreted to predict high testosterone concentrations in males during bouts of reproductive aggression, regardless of other important social factors that might also affect relations between androgens and behaviour in social mammals. Goymann et al. (2003) showed that male spotted hyaenas defending a female had higher concentrations of plasma T than did nondefending males in the Serengeti. We report here a similar result in Talek hyaenas, in that immigrant males had higher concentrations of fA when showing reproductive aggression and courting than when they were not showing reproductive aggression. However, our results are not consistent with Goymann et al.'s (2003) conclusion that defending males have higher concentrations of T because of higher interaction rates with other males. If higher interaction rates with other males alone were enough to raise androgen concentrations, we should have detected significantly higher concentrations of faecal androgens in males showing nonreproductive aggression than in males showing no aggressive behaviour. Instead, our results indicate that the context in which aggression occurs is important. Our results also suggest that male–male interactions over a female do not constitute a particularly challenging situation for male hyaenas. Males rarely fought over females, aggression intensities were low and consisted mostly of low-level threats between males, and the outcome of any male–male interaction was nearly perfectly predictable because of the stable social hierarchy and the queuing system among immigrants.

In their efforts to apply the challenge hypothesis to spotted hyaenas, Goymann et al. (2003) acknowledged the possibility that T concentrations were elevated in male hyaenas defending prospective mates because of their proximity to females. However, male–female interactions were not investigated in that study. The present work directly addressed this possibility by investigating whether the main social challenge for a male spotted



**Figure 4.** Correlation between faecal androgen concentrations in immigrant males and their average association index (AI) with (a) attractive females and (b) lactating females.  $N = 69$  faecal samples from 22 males.

hyaena is male–male competition or interacting with dominant females. Although we did find a significant relation between reproductive aggression and androgens, most males that showed reproductive aggression were also concurrently engaged in active courtship. Males that showed only courtship also had elevated androgen concentrations. These results demonstrate that interacting with females is associated with elevated androgen concentrations, independent of whether a male is also engaging in defensive aggressive interactions with other males over access to the females, thus supporting our hypothesis that interacting with females represents a substantial challenge to adult immigrant males. It was obvious upon observation that spotted hyaena males were often conflicted between tendencies to approach and flee during interactions with dominant females. When males were interested in a female, they sometimes engaged in brief running mounts and awkward dismounts, as well as masturbation next to a female. Males may need to reach some threshold of sexual stimulation to overcome this approach–avoidance conflict so as to actively court and eventually mate with a female.

The challenge hypothesis has been a useful starting point for formulating predictions concerning androgen–behaviour relationships in male vertebrates. However, the results presented here, as well as in other studies (Creel et al. 1993; Peters et al. 2001; Lynch et al. 2002; Moore et al. 2004), suggest that the hypothesis may be limited in its direct application to social mammals and some birds. The challenge hypothesis appears to lack predictive power for patterns in androgen responsiveness to challenge in species with low ratios of paternal care to male–male aggression (Figure 4.7 in Wingfield et al. 2000). This observation suggests that factors influencing male fitness other than direct male–male competition modulate hormone–behaviour relations in these species. Instead of attempting to modify the challenge hypothesis for application to complex social systems, we suggest that future research should test the influence of additional variables that may affect hormone–behaviour relations in free-living animals, including alternative reproductive tactics and male–female interactions.

We found that degree of association with attractive females positively influenced fA concentrations in immigrant male hyaenas. The reproductive state of the females with which a male associated was clearly important, because males associating closely with females in the month before conception had greater fA concentrations than did males associating with lactating females. An endocrine response of a male to a female may help to synchronize reproductive behaviour and physiology between the sexes when mating opportunities are unpredictable. Our findings suggest that, in spotted hyaenas, androgen concentrations are increased by interaction with attractive females, and that this increased androgen might then function to facilitate courtship and sexual behaviour, as well as occasional aggressive defence of females (Moore 1983; Wingfield et al. 1987, 1990; Beletsky et al. 1995; Balthazart et al. 1996). Although we cannot rule out the possibility that high concentrations of androgens may lead males to associate with females, limited

longitudinal data documenting variation in hormones and behaviour among males in the study clan cast doubt on this idea.

We were unable to investigate the relation between fA concentrations in adult natal males and the intersexual association patterns involving these males, because we had too few faecal samples from adult natal males in the months before known conceptions. However, we found no relation between courtship or reproductive aggression and fA concentrations in adult natal males, but we did find such relations in immigrant males. The results presented here, as well as those by Holekamp & Smale (1998) and Holekamp & Sisk (2003), strongly support the idea that adult males that have not yet dispersed differ both behaviourally and physiologically from males that have dispersed and immigrated into a new clan, and should be considered separately from immigrant males. East & Hofer (2002) and Goymann et al. (2003) maintain that some natal male hyaenas in the Serengeti never disperse. Instead, these natal males reportedly drop in social rank to the top of the immigrant male hierarchy in their natal clans and begin competing with immigrants for females. These nondispersing natal males submit to all adult females, including those of lower maternal rank, in all contexts (M. L. East, personal communication). Although some adult natal males in the Talek clan do show reproductive aggression and court females, they all do so at lower rates than do immigrant males (Holekamp & Smale 1998; this study). The six adult natal males for which we calculated rates of reproductive aggression and courtship were aggressive towards, but were never seen to submit to, lower-ranking females in contexts other than courting, such as feeding. Thus, adult natal males in the Talek clan do not appear to fall in social rank, as they are sometimes reported to do in the Serengeti. This discrepancy suggests a fascinating difference between the Talek and Serengeti populations, that males in one population but not the other occasionally adopt an alternative male strategy.

Szykman et al. (2001) found that immigrant male spotted hyaenas associate most closely with females that are most likely to be fertile, and we have shown that male androgen concentrations increase with this association. However, Szykman (2001) also examined the strength of associations between females and the males known to sire litters born to these females, and found that known sires varied greatly in how tightly they associated with their future mates during the month preceding conception. This result suggests that immigrant male hyaenas may adopt alternative reproductive tactics. If elevated androgen concentrations are costly to male hyaenas, as they are reported to be in other vertebrate species (Folstad & Karter 1992; Wedekind & Folstad 1994), then only males that can afford the cost of associating closely with females are likely to do so. Another potential cost to male hyaenas of associating closely with breeding females is the energetic cost of having these dominant females monopolize food resources. In response to these potential costs, alternative reproductive tactics in the male spotted hyaena may be mediated by variation in androgenic and stress hormones.

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