

LETTERS

Rank-related maternal effects of androgens on behaviour in wild spotted hyaenas

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Within any hierarchical society, an individual's social rank can have profound effects on its health and reproductive success^{1,2}, and rank-related variation in these traits is often mediated by variation in endocrine function². Maternal effects mediated by prenatal hormone exposure are potentially important for non-genetic inheritance of phenotypic traits related to social rank³, and thus for shaping individual variation in behaviour and social structure. Here we show that androgen concentrations in wild female spotted hyaenas (*Crocuta crocuta*) are higher during late gestation in dominant females than in subordinate females. Furthermore, both male and female cubs born to mothers with high concentrations of androgens in late pregnancy exhibit higher rates of aggression and mounting behaviour than cubs born to mothers with lower androgen concentrations. Both behaviours are strongly affected in other mammals by organizational effects of androgens⁴, and both have important effects on fitness in hyaenas. Therefore, our results suggest that rank-related maternal effects of prenatal androgen exposure can adaptively influence offspring phenotype in mammals, as has previously been shown to occur in birds. They also suggest an organizational mechanism for the development of female dominance and aggressiveness in spotted hyaenas, traits that may offset the costs of extreme virilization.

Maternal effects, which occur when maternal phenotype influences offspring phenotype independent of offspring genotype, permit environmental conditions experienced by the mother to affect offspring phenotype, potentially enhancing offspring fitness⁵. In oviparous vertebrates, maternally derived androgens in eggs mediate growth and behaviour in offspring^{3,6–8}. Importantly, the social environment of a laying bird (for example, social rank and aggressive interactions) can influence the amount of androgen transferred to its eggs^{9,10}. Here we report findings from a long-term study of free-living spotted hyaenas suggesting that the social status of a pregnant female mammal can similarly affect androgen exposure of her young *in utero* and thereby modify offspring phenotype.

The society of the spotted hyaena is structured by a rigid linear dominance hierarchy, and an individual's social rank determines its priority of access to resources as well as its reproductive success^{11–13}. Within a social group, female hyaenas therefore vary considerably with respect to condition, depending on the social ranks they 'inherit' from their mothers^{12,14}. Female spotted hyaenas are generally larger than males, and they exhibit strongly masculinized genitalia through which it is uniquely difficult to give birth¹⁵. Females are also more aggressive than males¹⁶ and socially dominant to them¹¹. Large body size and aggressiveness in female hyaenas seem to be linked to genital masculinization by common endocrine or epigenetic mechanisms, although how this linkage occurs is unknown¹⁷. Except during pregnancy, female hyaenas have lower levels of circulating testosterone than do adult males¹⁸, indicating that activational effects of androgens cannot account for the sex difference in aggressiveness

documented in this species. However, the ovaries of pregnant female hyaenas produce large quantities of androgenic steroid hormones during the second half of gestation, and these cross the placenta to reach the developing fetus^{19,20}. Thus, hyaena cubs of both sexes are exposed to high concentrations of androgens during fetal life. A number of sexually dimorphic behaviours exhibited by juvenile mammals are known to be shaped by prenatal androgen exposure, and in particular, aggression and sexual play are affected by organizational effects of androgens in a wide array of mammals²¹.

Here we tested the hypothesis that prenatal exposure of cubs to

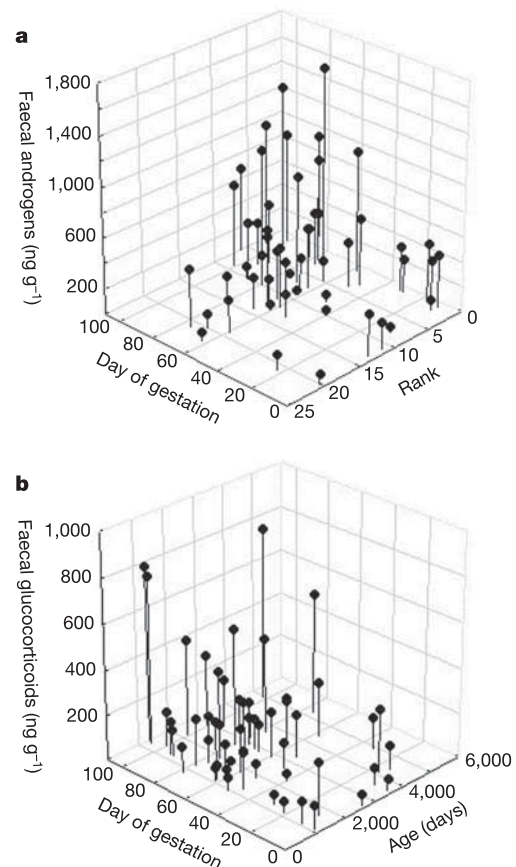


Figure 1 | Maternal hormones during gestation in wild spotted hyaenas. **a**, The graph shows plots of social rank, day of gestation and faecal androgen metabolite concentrations. **b**, The graph shows plots of maternal age, day of gestation and faecal glucocorticoid concentrations. The highest social rank possible is 1.

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androgens affects offspring phenotype in hyaenas. We first investigated patterns in faecal androgen concentrations throughout gestation in pregnant females. Faecal androgen concentrations increased as gestation progressed (whole model $R = 0.61$, $F_{3,49} = 9.62$, $P = 0.004$; day of gestation partial $R = 0.45$, $P < 0.001$; Fig. 1a), and high-ranking females had higher faecal androgen concentrations than did low-ranking females during pregnancy (rank partial $R = -0.46$, $F_{1,49} = 13.26$, $P < 0.001$). Female age was not associated with variation in faecal androgen concentrations during pregnancy ($F_{1,49} = 0.71$, $P = 0.41$).

Because social rank may also influence stress in pregnant female hyaenas, and stress experienced by female mammals during pregnancy can influence offspring development via elevated glucocorticoids²², we investigated the role of faecal glucocorticoid concentrations as a covariate. The multiple regression model for faecal glucocorticoid concentrations was significant (whole model $R = 0.62$, $F_{3,49} = 10.43$, $P < 0.001$; Fig. 1b), and day of gestation explained a large proportion of the variance in faecal glucocorticoid concentrations, with faecal glucocorticoids increasing in the second half of gestation (partial $R = 0.57$, $F_{1,49} = 23.63$, $P < 0.001$). Social rank had no effect on faecal glucocorticoid concentrations in pregnant females ($F_{1,49} = 0.63$, $P = 0.43$), but older females had higher faecal glucocorticoid concentrations than younger females during gestation (partial $R = 0.38$, $F_{1,49} = 8.26$, $P = 0.006$). Thus, although several maternally associated variables influenced faecal glucocorticoid concentrations, maternal rank did not predict faecal glucocorticoid concentrations in pregnant female hyaenas.

In other mammals, day of gestation strongly affects patterns of fetal masculinization induced by androgenic hormones, with behavioural masculinization occurring later than genital masculinization²³. Because our outcome variables here were behavioural, we next inquired whether maternal rank or day of gestation affected faecal hormone concentrations in female hyaenas sampled during the second half of gestation. We also tested for relationships among litter composition, rate of maternal aggression during late pregnancy and maternal hormones by including the two former variables as covariates. Using these data, a multiple regression model of faecal androgen concentrations was still significant (whole model $R = 0.69$, $F_{5,15} = 3.08$, $P = 0.038$). High-ranking females had higher faecal androgen concentrations than low-ranking females (partial $R = -0.49$, $F_{1,15} = 6.24$, $P = 0.025$; Fig. 2), but here day of gestation was not significant ($F_{1,15} = 3.16$, $P = 0.10$). Furthermore, we found no relationship between either litter composition ($F_{2,15} = 0.22$, $P = 0.81$; Table 1) or maternal aggression rate during late pregnancy ($F_{1,15} = 0.51$, $P = 0.48$) and maternal faecal androgen concentrations. The multiple regression model for faecal glucocorticoid concentrations was significant (whole model $R = 0.83$, $F_{4,16} = 8.82$, $P < 0.001$); day of gestation influenced faecal glucocorticoid concentrations (partial $R = 0.75$, $F_{1,16} = 28.89$, $P < 0.001$), but age of

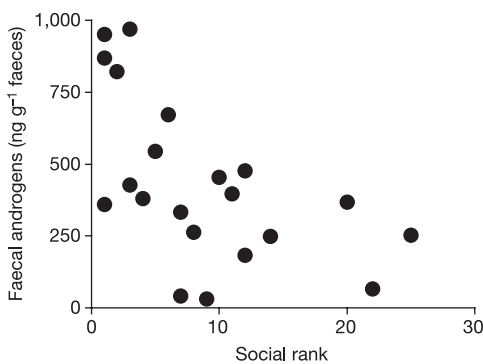


Figure 2 | The relationship between faecal androgens and social rank in pregnant female spotted hyaenas during the second half of gestation. The highest social rank possible is 1.

Table 1 | The effect of litter composition on maternal hormones in spotted hyaenas

Litter composition	Faecal androgen (ng g ⁻¹)	Faecal glucocorticoid (ng g ⁻¹)	N
Female	447.28 ± 80.97	263.66 ± 95.98	7 (4 twins, 3 single)
Mixed	429.38 ± 117.99	113.09 ± 25.60	8
Male	436.53 ± 34.42	369.28 ± 126.53	6 (2 twins, 4 single)

Values are mean ± standard error faecal androgen and faecal glucocorticoid concentrations in relation to litter composition during the second half of gestation in wild spotted hyaenas, which generally give birth to litters of one or two cubs.

mother was not significant ($F_{1,16} = 0.512$, $P = 0.48$). There was no effect of social rank ($F_{1,16} = 0.20$, $P = 0.66$) or litter composition ($F_{1,16} = 0.24$, $P = 0.63$; Table 1) on maternal faecal glucocorticoid concentrations during the second half of gestation.

Rates of aggression and mounting in cubs were positively related to maternal faecal androgen concentrations during the second half of gestation (Fig. 3). The analysis of covariance (ANCOVA) for cub mounting rate (whole model $R = 0.681$, $F_{7,27} = 6.48$, $P < 0.001$; Fig. 3a) revealed significant effects of cub sex ($F_{1,28} = 8.13$, $P = 0.008$) and maternal faecal androgen concentration ($F_{1,28} = 7.91$, $P = 0.009$), and a significant interaction between these two variables (sex × faecal androgen interaction $F_{1,28} = 17.97$, $P < 0.001$). Male cubs generally showed higher rates of play mounting than did female cubs. Maternal faecal androgen concentrations were significantly and positively related to mounting rates in both sexes, but this relationship was more robust in males than females. Neither maternal faecal glucocorticoid nor maternal rank itself significantly influenced mounting rates of cubs (faecal glucocorticoid residuals $F_{1,28} = 0.86$, $P = 0.36$; rank $F_{1,28} = 0.16$, $P = 0.69$).

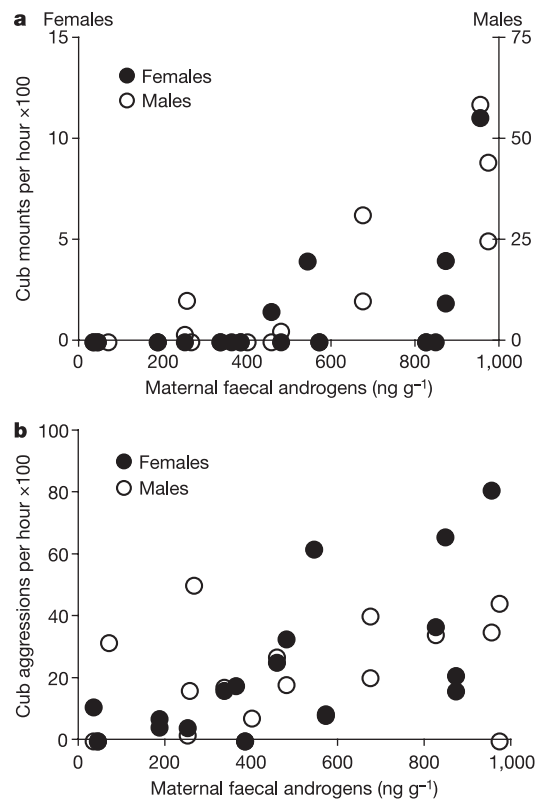


Figure 3 | Maternal androgens and cub behaviour. The relationship between maternal androgens measured during the second half of gestation and rates of mounting (a) and aggression (b) of male and female hyaena cubs aged 2–6 months. Values for mounting and aggressive behaviours are multiplied by 100 for ease of data analysis and presentation.

Similarly, we found a relationship between maternal faecal androgen concentration and cub aggression that was not directly affected by maternal rank or any other variable explored. The ANCOVA applied to the aggression data was significant (whole model $R = 0.64$, $F_{6,28} = 3.25$, $P = 0.015$; Fig. 3b). Rates of cub aggression did not vary with cub sex ($F_{1,28} = 2.745$, $P = 0.11$), maternal rank ($F_{1,28} = 0.34$, $P = 0.57$), maternal aggression during early lactation ($F_{1,28} = 2.70$, $P = 0.12$), or maternal faecal glucocorticoid residuals ($F_{1,28} = 0.99$, $P = 0.33$). However, there was a significant positive influence of maternal faecal androgen concentration on cub aggression rates ($F_{1,28} = 7.37$, $P = 0.011$), and a trend towards interaction between cub sex and maternal faecal androgen ($F_{1,28} = 8.08$, $P = 0.08$).

An extensive body of research indicates that the prenatal sex hormone environment is extremely important with respect to the morphological and behavioural development of mammalian offspring. However, to our knowledge this is the first study to demonstrate a relationship between the social rank of a free-ranging mammal and its androgen profile during pregnancy, as well as to show that this variation is related to offspring behavioural phenotype, thus suggesting a new mechanism for the transfer of status-related traits from mother to offspring via prenatal hormone exposure. Our results also offer the first definitive link between prenatal androgens and aggression in female spotted hyaenas, and they support the hypothesis that selection for aggression under intense feeding competition was the original selective pressure leading to enhanced female aggressiveness and other 'masculinized' features of female hyaenas. Aggressiveness in female spotted hyaenas should be adaptive because feeding competition in this species is very intense, and the females best able to displace conspecifics from food at ungulate kills experience the highest reproductive success^{12,13}.

Social dominance and genital masculinization in females make sex extremely challenging for male hyaenas²⁴, so males with enhanced mating ability should also be favoured by natural selection. Orienting correctly on the female during mounting is critical for the male to achieve intromission during sexual interactions, and the amount of practice a male obtains early in life may profoundly affect its lifetime reproductive success. Male hyaenas exposed to anti-androgens *in utero* experience difficulties when attempting to mate in captivity²⁵, and we expect that high-ranking sons exposed to relatively high prenatal androgen concentrations in the wild should be more successful at siring cubs than sons exposed to lower prenatal androgen concentrations. Thus, prenatal androgen exposure in both sexes may have adaptive consequences that offset the costs of female virilization in this species.

METHODS

Study population. Data were collected from hyaenas in one large, stable clan in the Masai Mara National Reserve, Kenya, from July 1993 to July 2002. This clan has been studied since 1979, and details on general methods can be found in earlier publications¹⁸. We used critical incident sampling²⁶ of all agonistic behaviour observed, and adult female social ranks were determined based on a matrix of dyadic agonistic interactions as described previously¹⁴. Age and current social rank of each female at each time of sampling were known from long-term records.

Faecal sample processing and assays. We collected 53 faecal samples from a total of 43 pregnancies of 27 adult female hyaenas. Day of gestation for a given faecal sample was determined by back-calculating from the birth date of cubs¹⁴ resulting from each pregnancy. The number of days between the sample and the birth date was subtracted from 110 days, the length of the gestation period in spotted hyaenas.

Faecal samples were collected, stored frozen and assayed for faecal androgen metabolites using an enzyme immunoassay that uses a testosterone antibody that crossreacts with dihydrotestosterone and androstenedione¹⁸. Variation in faecal androgen concentrations parallels variation in circulating testosterone in this species¹⁸. The inter-assay coefficient of variation was 7.82%, and the intra-assay coefficient of variation was 9.4%. Faecal extracts were also assayed for glucocorticoid metabolites with a modified version of an assay previously

validated for use with spotted hyaena faeces²⁷. Briefly, reconstituted faecal extracts were diluted 1:20 in steroid diluent and measured in duplicate for immunoreactive glucocorticoids by radioimmunoassay kit. This assay uses a corticosterone antibody (ICN Biomedicals) that crossreacts with the major glucocorticoid metabolites present in the faeces of spotted hyaenas²⁷. The inter-assay coefficient of variation was 11.11%, and the intra-assay coefficient of variation was 4.5%.

Behavioural data. Behavioural data were collected using critical incident sampling²⁶. Aggression rates were calculated for each adult female by dividing the total number of aggressions emitted by the total amount of time observed during the second half of gestation and the first six months of lactation. Aggression included the following behaviours: lunge, snap, bite, chase, displace, push, stand over, and intention movement to bite^{11,14}. Subject animals for behavioural analyses of offspring included all cubs born to females for which we had faecal samples from the second half of gestation, and that survived to at least 6 months of age. This included 35 cubs in eight mixed-sex twin litters, two male–male twin litters, four female–female twin litters, four singleton males and three singleton females. For each cub, we recorded all aggressions that were directed towards non-littermates, and all mounts of conspecifics. Cubs directed aggressive behaviour towards other den-dwelling cubs and older juveniles, and occasionally towards adults. Aggressive behaviour occurred in the context of competition over access to solid food as well as in other contexts. Play mounting involved one cub approaching another animal from behind, rising up on the hind legs and placing the forepaws on the other individual's back with a posture like that exhibited by adult males during copulation. For each cub, hourly rates of mounting and aggression were calculated by dividing the numbers of observed behaviours of each type by the number of hours each animal was observed during the four-month interval when cubs were 2–6 months of age. At this age, cubs have not yet attained their maternal social ranks¹⁴, so patterns of aggressive behaviour among cubs are not yet shaped by the rank relationships existing among their mothers. Cubs were observed for an average of 36.1 ± 3.8 h during this period.

Statistical analysis. To investigate the relationship between social rank and faecal steroid concentrations among pregnant females we used mixed model multiple regressions. Owing to an unbalanced design with some repeated measures, we also included individual hyaena as a random effect variable. Individual hyaena did not have a significant effect and was therefore removed from the model. We also redid these two analyses using a data set consisting of only a single random sample from each female ($N = 27$) to determine the robustness of the significant effects found in the larger data set. In these analyses, results were consistent. Faecal androgen: whole model $R = 0.62$, $F_{2,24} = 7.39$, $P = 0.003$; social rank partial $R = -0.35$, $P = 0.049$; day of gestation partial $R = 0.41$, $P = 0.02$. Faecal glucocorticoid: whole model $R = 0.70$, $F_{2,24} = 11.29$, $P < 0.001$; maternal age partial $R = 0.47$, $P = 0.016$; day of gestation partial $R = 0.62$, $P < 0.001$. We used ANCOVA to investigate variables influencing maternal hormones during the second half of gestation only, and we investigated the relationship between maternal hormones and cub behaviour with separate ANCOVAs for aggression and play mounting. Data met assumptions for the used tests and alpha was set at 0.05.

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