

Exposure to naturally circulating androgens during foetal life incurs direct reproductive costs in female spotted hyenas, but is prerequisite for male mating

C. M. Drea^{1*}, N. J. Place¹, M. L. Weldele¹, E. M. Coscia¹, P. Licht²
and S. E. Glickman^{1,2}

¹Department of Psychology, and ²Department of Integrative Biology, University of California at Berkeley, Berkeley, CA 94720, USA

Among all extant mammals, only the female spotted hyena (*Crocuta crocuta*) mates and gives birth through the tip of a peniform clitoris. Clitoral morphology is modulated by foetal exposure to endogenous, maternal androgens. First births through this organ are prolonged and remarkably difficult, often causing death in neonates. Additionally, mating poses a mechanical challenge for males, as they must reach an anterior position on the female's abdomen and then achieve entry at the site of the retracted clitoris. Here, we report that interfering with the actions of androgens prenatally permanently modifies hyena urogenital anatomy, facilitating subsequent parturition in nulliparous females who, thereby, produce live cubs. By contrast, comparable, permanent anatomical changes in males probably preclude reproduction, as exposure to prenatal anti-androgens produces a penis that is too short and has the wrong shape necessary for insertion during copulation. These data demonstrate that the reproductive costs of clitoral delivery result from exposure of the female foetus to naturally circulating androgens. Moreover, the same androgens that render an extremely unusual and laborious process even more reproductively costly in the female are apparently essential to the male's physical ability to reproduce with a normally masculinized female.

Keywords: masculinization; androgen; sexual differentiation; reproductive success; spotted hyena

1. INTRODUCTION

(a) *Female masculinization and reproduction in the spotted hyena*

The female spotted hyena (*Crocuta crocuta*) presents the most 'masculinized' external genitalia of any female mammal (Watson 1877; Matthews 1939; Davis & Story 1949; Neaves *et al.* 1980; Frank *et al.* 1990). There is no external vagina, as the labia are fused to form a pseudosrotum and the clitoris is hypertrophied and fully erectile, resembling the male's penis in size and structure. This pseudo-penis is traversed to its tip by a central urogenital canal, through which the female urinates, copulates and gives birth. Despite increased width and elasticity over males (Frank *et al.* 1990; Glickman *et al.* 1992; Drea *et al.* 1998), the narrow opening or urogenital meatus in the clitoris of adult, nulliparous females must tear during parturition to allow passage of a comparatively large cub (Frank & Glickman 1994). For example, the meatus of virgin females measures *ca.* 2.5 cm in diameter when gently distended (Glickman *et al.* 1992), but a typical foetus has a head diameter of *ca.* 7 cm. The protracted labour required to expel the foetus leads to high rates of neonatal mortality in first litters, usually owing to foetal anoxia (Frank *et al.* 1995; figure 1).

Whereas first births are exceptionally difficult and reproductively costly (Frank & Glickman 1994; Frank *et al.*

et al. 1995), copulation through a peniform clitoris is merely mechanically awkward. Important sex differences in 'phallic' anatomy probably function to facilitate copulation (and parturition), including the specific positioning of the urogenital canal in the clitoris to allow expansion and robust development of the retractor clitoridis muscle (Watson 1877; Matthews 1939; Neaves *et al.* 1980; Cunha *et al.* 2003). Although a sexually receptive female draws her clitoris into the abdomen to allow penetration by the male, her clitoral opening is well anterior to the position typically occupied by an external vagina (Drea *et al.* 1999). Thus, when mounting a female, males have considerable difficulty locating this opening and achieving intromission (Schneider 1952; Kruuk 1972; Drea *et al.* 1999; Fenstemaker *et al.* 1999).

(b) *Potential role of androgens in masculinizing female genitalia*

According to contemporary understanding of sexual differentiation, the extreme virilization of the female spotted hyena should be due to androgens acting on the female foetus at critical stages of development (Matthews 1939; Racey & Skinner 1979; Glickman *et al.* 1987, 1992; Yalcinkaya *et al.* 1993; Licht *et al.* 1998). Prior research from our laboratory showed that testosterone, produced by the placenta through metabolic conversion of maternal Δ^4 androstenedione (a prohormone of ovarian origin), is transferred to foetuses of both sexes via the umbilical circulation (Yalcinkaya *et al.* 1993; Licht *et al.* 1998). Such transfer begins at an early stage of gestation and continues until parturition. Thus, a source of androgen is available

* Author and address for correspondence: Department of Biological Anthropology and Anatomy, 08 Biological Sciences Building, Box 90383, Duke University, Durham, NC 27708-0383, USA (cdrea@duke.edu).



Figure 1. Parturition in a normal, nulliparous female spotted hyena, resulting in a stillborn cub. The typically large cub is still in the amniotic sac, with the head adjacent to the torn clitoral meatus. The placenta and remnants of umbilical cord are visible within the stretched clitoral opening. Photograph by S. E. Glickman.

to the developing female foetus and could potentially account for her male-like external genital morphology.

(c) *Interfering with the actions of prenatal androgens*

We recently tested this theory by treating pregnant spotted hyenas with a mixture of oral 'anti-androgens' (Drea *et al.* 1998). Anti-androgen treatment consisted of finasteride (0.6 mg kg^{-1} daily) and flutamide ($23\text{--}24 \text{ mg kg}^{-1}$ daily), delivered from as early as day 21 of a 110-day gestation. The former compound, a 5α -reductase inhibitor, prevents conversion of testosterone to dihydrotestosterone, which is traditionally assumed to be the critical androgen involved in formation of male genitalia (Siiteri & Wilson 1974; Wilson *et al.* 1981). The latter compound, an anti-androgen, blocks the actions of all active androgens at the receptor level.

Oral administration of the finasteride/flutamide mixture to pregnant hyenas, for prolonged periods, produced litters in which both male and female infants (hereafter termed 'experimental' animals) retained prominent phallic and scrotal structures (Drea *et al.* 1998). Thus, experimental female infants still lacked an external vagina and displayed a penile clitoris. Although such prenatal treatment did not produce the typical 'feminization' seen in other mammalian species (Neri 1977; Thornton *et al.* 1991; Imperato-McGinley *et al.* 1992; Herman *et al.* 2000), there were significant 'feminizing' effects on external genital morphology provided that 'femininity' is defined in terms of the normal sex differences between male and female spotted hyenas (Drea *et al.* 1998).

Prenatal anti-androgen treatment modified the appearance of the infant male glans, producing a penis with the essential morphological characteristics of the normal female hyena clitoris (i.e. a shorter, thicker organ, with a rounded rather than angular contour and a larger, more elastic opening at the tip). Similarly, the experimental infant female displayed a still shorter and thicker clitoris, with an exceptionally large and highly elastic opening at the tip (the urogenital meatus). Thus, blocking androgens *in utero* changed both the relaxed, static size and elasticity of the clitoral opening. As experimental infants studied for

the first six months of life showed 'feminization' of external genital morphology compared with age-matched controls, it is clear that urogenital development of male and female foetuses is influenced by circulating androgens (Drea *et al.* 1998).

(d) *Testing the effects of prenatal anti-androgen treatment on adult reproductive success*

Our present goal was to extend our observations of experimental hyena infants through sexual maturity and into adulthood, reporting on the later pattern of genital development and examining the consequences, if any, on reproductive success. Previously published data on these same subjects stopped at six months (Drea *et al.* 1998), where these data pick up. In particular, if a normal female hyena's exposure to endogenous androgens is associated with birthing difficulties during her first parturition, then feminization of the external genitalia following prenatal anti-androgen treatment should facilitate the birthing process. Although focused on the reproductive success of females, data emerged that are also relevant to the ability of males to successfully impregnate females.

2. METHODS

(a) *Subjects*

The animals were members of a captive colony housed socially at the Field Station for Behavioural Research, University of California, Berkeley. The experimental animals (four females and three males) were derived from five anti-androgen-treated pregnancies (for which the details of maternal anti-androgen regimens are provided in table 1 of Drea *et al.* (1998)). The control animals (26 females and 28 males) were derived from normal pregnancies over a span of 15 years. The sample size of control animals varies for different measures, at different ages, due to competing participation of subjects in other aspects of our research programme. All available data are reported for every age category. All animals were fed Nebraska Brand carnivore zoo diet and pork bones each morning, and fresh straw for bedding was routinely provided.

The animals were followed from six months (the juvenile period) through five years of age. During this time, shoulder height and body length measurements of females and males revealed similar growth patterns in control and experimental animals (table 1). Although sexual maturity occurs at *ca.* two years for males and three years for females (Matthews 1939; Kruuk 1972; Drea *et al.* 1999), first pregnancies are often delayed past sexual maturity due to colony management issues. Thus, the five-year period reflects the time required for all experimental females to give birth a first time. Similarly, 12 of the control females had 'normal' first pregnancies (other control females that underwent Caesarian section or clitoral episiotomy were excluded from our analyses of reproductive success).

(b) *Genital measurements*

Genital development was monitored during routine physical examinations conducted approximately biannually. The animals were measured following immobilization with intramuscular injections of ketamine ($4\text{--}6 \text{ mg kg}^{-1}$) and xylazine (1 mg kg^{-1}). Because of variation in scheduling, monthly ages were grouped into bins for data analysis and presentation (figures 2 and 3). Each bin represents the midpoint of gradually increasing time intervals. Thus, ages 6 and 9 represent one-month intervals; ages

Table 1. Sex- and age-related changes in mean body length and shoulder height in control versus experimental spotted hyenas. (As sample size for experimental (exp) females ($n = 4$) and males ($n = 3$) remains constant, n indicates the number of control (con) animals; measurements are expressed in mm with s.e.; t , Student's t -test statistic, with associated degrees of freedom; and p , probability.)

subject	body length					shoulder height				
	n	con (\pm s.e.)	exp (\pm s.e.)	t	p	n	con (\pm s.e.)	exp (\pm s.e.)	t	p
female										
six months	16	897 (16)	903 (30)	$t_{18} = 0.18$	0.86	16	552 (13)	560 (9)	$t_{18} = 0.32$	0.75
24 months	14	1257 (16)	1259 (20)	$t_{16} = 0.06$	0.95	14	790 (12)	793 (11)	$t_{16} = 0.16$	0.88
male										
six months	19	888 (10)	922 (21)	$t_{20} = 1.24$	0.23	19	567 (10)	590 (16)	$t_{20} = 0.91$	0.37
24 months	13	1221 (10)	1257 (19)	$t_{14} = 1.57$	0.14	14	787 (7)	814 (6)	$t_{15} = 1.66$	0.12

12 and 18 represent three-month intervals (i.e. 11–13 and 17–19); and ages 24 and 30 represent five-month intervals (i.e. 22–26 and 28–32). Whereas measurements of genital morphology are taken throughout a male's lifetime, females are no longer measured postpartum owing to the clitoral tearing that accompanies first births (figure 1). Thus, our upper age limit is the last time when all experimental females were still nulliparous.

The same genital measurements reported in Drea *et al.* (1998) were also obtained in the present experiment and in the same manner; however, we report here on a subset of those measurements. In both males and females, we measured the circumference of the relaxed organ and the length of the taut 'phallus' (stretched with a force of 400 g). As it is not possible to obtain the latter measure using the same tension at all ages, the earliest age bins are not represented for this measurement. Additional measurements of interest in females include the length of the relaxed and stretched urogenital meatus. The latter measurement was obtained while gently distending the meatus with a force of 100 g (Glickman *et al.* 1992). Whereas this standard tension challenges the elasticity of the meatus in control females, it underestimates the elasticity and maximum diameter of the meatus in experimental females (Drea *et al.* 1998). Lastly, we also noted distal penile morphology, specifically the contour of the erect glans. As control and experimental animals were of equal body size (table 1), no correction factor was required for these genital measurements.

The overall mean values plotted in figures 2 and 3 are based on all hyenas measured per age bin, with individual means calculated for any animals sampled multiple times within a given interval (sample sizes are provided in the legends). However, after assumptions about homogeneity of variance and normality about the mean were satisfied, we performed conservative analyses of variance (ANOVA) for repeated measures using only the subset of animals that were represented at every time point. In all cases, the subset of subjects with repeated measures displayed the same pattern as the larger population.

(c) *Mating, pregnancies and first deliveries*

Starting in early adulthood, females were temporarily removed from their social group and repeatedly paired with control and/or experimental males to achieve timed copulation. Mating behaviour was observed and recorded on videotape (E. M. Coscia, unpublished data). Mated females were subsequently immobilized and blood was drawn from the jugular vein using a sterile 22 gauge needle-vacutainer system. The blood was then centrifuged at 1000 g for 5 min and the plasma drawn and frozen

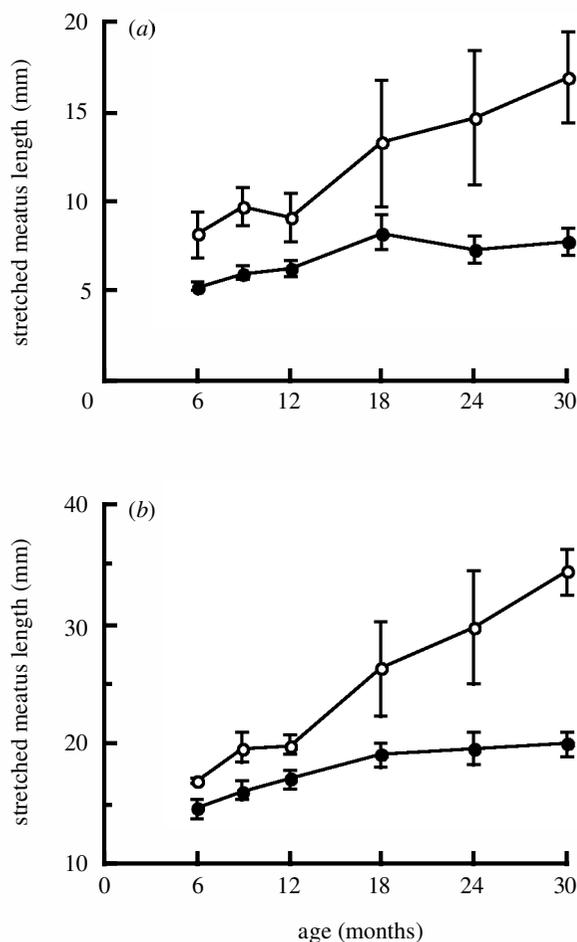


Figure 2. Length (mean \pm s.e. in mm) of the (a) relaxed and (b) stretched urogenital meatus from 6 to 30 months of age in female spotted hyenas exposed prenatally to androgen blockers (open circles) compared with age-matched controls (filled circles). Plotted values for both measures include the four experimental females at all ages and eight, nine, nine, nine, seven and nine control females at each consecutive age period, respectively. A repeated-measures ANOVA for control ($n = 6$) and experimental ($n = 4$) females revealed significant effects of treatment ($F_{1,8} = 11.36, p < 0.01$) and age ($F_{5,40} = 7.81$ and $p < 0.001$), as well as a significant interaction between treatment and age ($F_{5,40} = 4.10, p < 0.005$) on relaxed meatus length. Likewise, there were significant effects of treatment ($F_{1,8} = 10.27$ and $p < 0.025$) and age ($F_{5,40} = 22.59, p < 0.001$), as well as a significant interaction between treatment and age ($F_{5,40} = 9.24, p < 0.001$) on stretched meatus length.

for subsequent radioimmunoassay (see § 2d). Pregnancies were initially identified by elevated plasma progesterone concentrations and later confirmed by ultrasound. Prior to parturition, the females were isolated in covered enclosures (2 m × 2 m) and monitored using a wide-angled video camera. Parturition was either observed directly or recorded on tape, so the fate of cubs (i.e. survival or stillbirth), according to their birth order, was known for all litters. Data on female reproductive success are presented for first pregnancies only.

(d) Steroid assays

Because oestrogen affects the width and elasticity of the urogenital meatus (Glickman *et al.* 1992, 1998) and because hormonal profiles of the female spotted hyena are markedly altered during pregnancy (Licht *et al.* 1992, 1998), we also examined circulating concentrations of maternal steroids. We sampled control ($n=7$) and experimental ($n=4$) females during late stages of pregnancy (i.e. days 91–110 of gestation). We performed radioimmunoassays for progesterone (see § 2c), oestrogen, testosterone and Δ^4 androstenedione, as previously described (Licht *et al.* 1982, 1992).

3. RESULTS

(a) Genital development, maternal hormones and success of initial births in females

Significant differences in measurements of genital morphology between control and experimental hyenas were evident at every age examined. Notably, the urogenital meatus in experimental females prior to parturition was significantly longer and more elastic than that of control females (figure 2). Moreover, the clitoris of experimental females was shorter and thicker than that of age-matched controls (figure 3*a,b*). Thus, at an age when females in our colony give birth for the first time (*ca.* 30 months), the clitoris was shorter ($t_{11}=2.85$, $p<0.02$), thicker ($t_{21}=4.70$, $p<0.001$) and the opening more elastic ($t_{11}=7.37$ and $p<0.001$) in females exposed prenatally to anti-androgens.

There were no significant differences in steroid concentration (ng ml^{-1}) between control and experimental females during the period prior to parturition in mean (\pm s.e.) oestrogen (control: 1.75 ± 0.32 versus experimental: 2.45 ± 0.35 ; $t_9=1.39$, $p=0.20$), testosterone (control: 2.48 ± 0.42 versus experimental: 3.86 ± 0.80 , $t_9=1.70$, $p=0.12$) or Δ^4 androstenedione (control: 6.62 ± 1.23 versus experimental: 6.25 ± 0.84 , $t_9=0.20$, $p=0.84$).

As for female reproductive success, the 12 pregnancies involving nulliparous, control dams produced 20 cubs, of which 11 were stillborn and one expired within 48 h (60% mortality). By contrast, the four pregnancies involving nulliparous, experimental dams produced seven cubs, all of which survived clitoral delivery (0% mortality). Total cub mortality in litters born to control females was significantly greater than in litters born to experimental females ($p<0.02$, Fisher's exact test). Whereas eight (67%) of the control females' litters resulted in the death of the first-born cub (figure 1), none of the first cubs to pass through a 'feminized' clitoris succumbed, not even one delivered in caudal presentation, which is a complication with normally lethal consequences in spotted hyenas (Frank & Glickman 1994). Despite the smaller

sample size, mortality of first-born cubs tended to be greater in control than experimental dams ($p=0.077$, Fisher's exact test). Whereas datapoints of the prior analysis are not entirely independent (as cubs within litters are derived from the same mother), datapoints in the latter analysis are independent.

(b) Genital development and mating potential in males

As with females, the 'feminization' effects previously reported in infants and juveniles (Drea *et al.* 1998) also persisted in males into adulthood. Thus, experimental males had shorter and thicker penises than age-matched controls (figure 3*c,d*). In addition, experimental males maintained a rounded rather than angular contour in the shape of their glans, evident during erections (see fig. 2*b* in Drea *et al.* 1998). At the age when males normally mate for the first time (24 months), experimental animals were equipped with a shorter ($t_{10}=4.72$, $p<0.001$) thicker ($t_{17}=2.96$, $p<0.01$) and more rounded penis than controls.

All three experimental males displayed erections and mounted experimental females, but no complete mating sequences (including intromission and ejaculation) were observed and no pregnancies ensued. The same set of experimental females all became pregnant, however, following mounting and mating with control males during a comparable time period.

4. DISCUSSION

Prenatal exposure to androgen blockers eliminated the cost of clitoral delivery in adult female spotted hyenas. Increased cub survival in experimental, nulliparous females is consistent with the hypothesis that naturally circulating androgens modify feminine clitoral morphology in a manner that results in a high percentage of stillbirths. This condition is alleviated by the continued gain in diameter and elasticity of the clitoral meatus through adolescence and into adulthood, and by the shorter organ resulting from prenatal anti-androgens. The latter could potentially house a larger birth canal, but that possibility is unclear at present. The critical importance of an enlarged meatus is illustrated by a reduction in cub mortality in parous females (i.e. those that have previously experienced clitoral stretching and tearing). As noted in a prior publication, 93% of cubs born to multiparous females survive under similar captive conditions (Frank *et al.* 1995). Likewise, two nulliparous females that had received a veterinary episiotomy, involving surgical enlargement of their clitoral meatus prior to delivery, both gave birth to live cubs. We argue that by approximating a functional episiotomy, the morphological changes in the external genitalia of experimental females facilitated first parturition, resulting in 100% cub survival.

During the late stages of a normal spotted hyena pregnancy, oestradiol, testosterone and dihydrotestosterone reach exceptionally high concentrations in maternal plasma, with androgens approaching the highest concentrations observed in adult male hyenas (Licht *et al.* 1992, 1998). However, there were no significant differences in steroid concentration between experimental and control females during the period prior to parturition. Thus, it

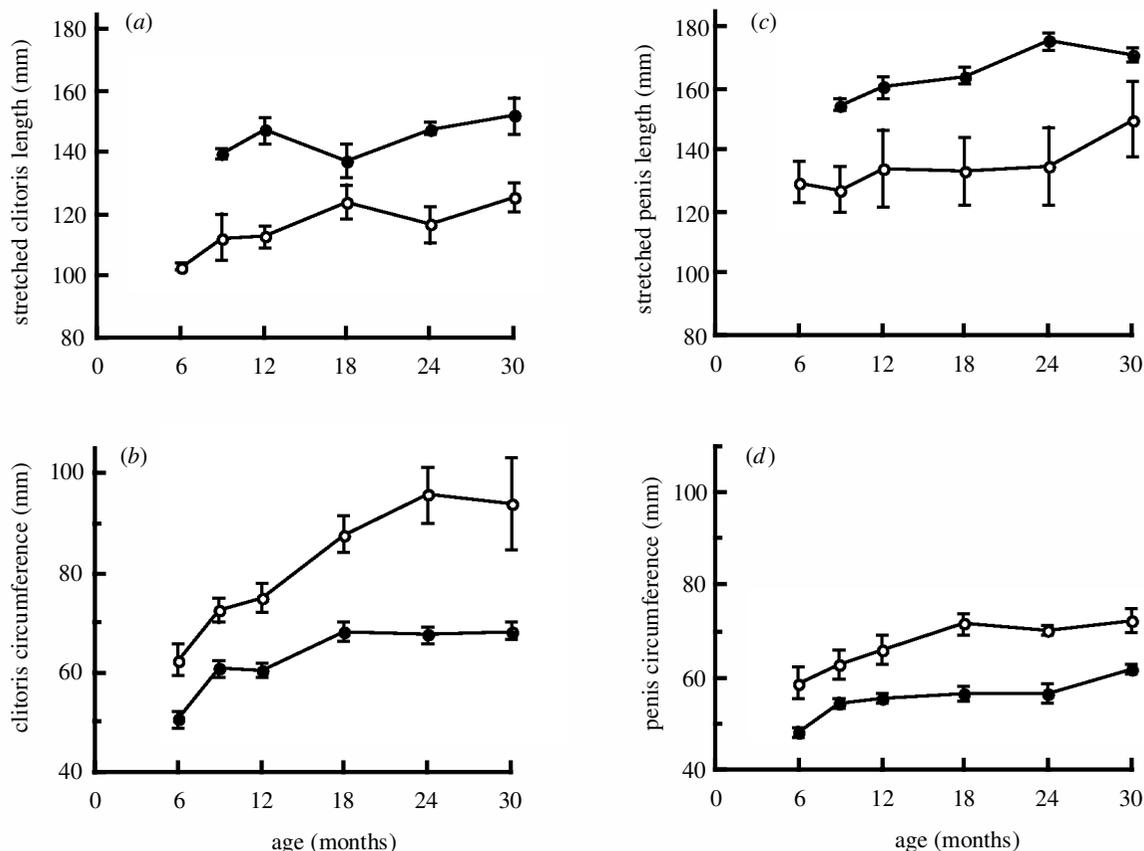


Figure 3. 'Phallic' development from 6 to 30 months of age in (a,b) female and (c,d) male spotted hyenas exposed prenatally to androgen blockers (open circles) compared with age-matched controls (filled circles). Measurements include (a,c) the length (mean \pm s.e. in mm) of the stretched 'phallus' and (b,d) the circumference (mean \pm s.e. in mm) of the relaxed 'phallus'. In (a,b), plotted values include the four experimental females at all ages. In (a), plotted values include zero, two, three, six, four and nine control females at each consecutive age period, respectively. A repeated-measures ANOVA for control ($n = 3$) and experimental ($n = 4$) females revealed significant effects of treatment ($F_{1,5} = 22.56, p < 0.005$) and age ($F_{3,15} = 9.41, p < 0.001$), as well as a significant interaction between treatment and age ($F_{3,15} = 0.96, p < 0.05$) on clitoral length from 12–30 months. In (b), plotted values include 19, 16, 18, 19, 17 and 19 control females at each consecutive age period, respectively. A repeated-measures ANOVA for control ($n = 8$) and experimental ($n = 4$) females revealed significant effects of treatment ($F_{1,10} = 17.82, p < 0.002$) and age ($F_{5,50} = 26.16, p < 0.001$), as well as a significant interaction between treatment and age ($F_{5,50} = 5.83, p < 0.001$) on clitoral circumference. In (c,d), plotted values include the three experimental males at all ages. In (c), plotted values include zero, six, eight, six, nine and 13 control males at each consecutive age period, respectively. A repeated-measures ANOVA for control ($n = 3$) and experimental ($n = 3$) males revealed significant effects of treatment ($F_{1,4} = 8.47, p < 0.05$) and age ($F_{4,16} = 9.05, p < 0.001$), but no interaction between treatment and age ($F_{4,16} = 1.88, p = 0.16$) on penis length between 9 and 30 months. In (d), plotted values include 25, 21, 23, 16, 16 and 23 control males at each consecutive age period, respectively. A repeated-measures ANOVA for control ($n = 12$) and experimental ($n = 3$) males revealed significant effects of treatment ($F_{1,13} = 10.32, p < 0.01$) and age ($F_{5,65} = 30.95, p < 0.001$), but no interaction between treatment and age ($F_{5,65} = 0.84, p = 0.52$) on penile circumference.

seems unlikely that an elevation in oestrogens accounts for the increased meatus elasticity of experimental females.

Clitoral delivery involves an unusual right-angled trajectory through the pelvic region. Consequently, the birth canal of a normal spotted hyena is both longer and more circuitous than that of similar-sized mammals (Frank & Glickman 1994; Frank *et al.* 1995). These structural constraints clearly contribute to the complicated and protracted labour in this species and probably account for some cases of maternal death associated with parturition (Glickman *et al.* 1993). Nevertheless, further 'masculinization' of a female spotted hyena's external genitalia, through prenatal exposure to testosterone (the result of placental conversion of maternal androstenedione) and dihydrotestosterone ultimately increases the mortality risk for that female's cubs.

Authors have previously proposed varying explanations for the evolution of 'masculinized' external genitalia in female spotted hyenas (reviewed in Glickman *et al.* 1993; Frank 1997). The majority have hypothesized that such masculinization was an incidental by-product of selection for raised foetal androgens (Gould 1981; Gould & Vrba 1982), stipulating that these androgens either facilitated adult competitive success during feeding at a kill (Hamilton *et al.* 1986), for example by mediating dominant social status (Frank 1997), or promoted successful neonatal aggression during competitive nursing (East *et al.* 1993). Others have provided alternative explanations that involve clitoral maculinization as a direct target of selection. These have related clitoral hypertrophy to the benefits of participation in meeting ceremonies (Kruuk 1972), vaginal closure to female control over mating (East *et al.*

1993) and genital mimicry to the potential advantages of reducing aggression from other females by presenting as males (Muller & Wrangham 2002).

Through hormonal manipulation, our previous studies (Drea *et al.* 1998; Glickman *et al.* 1998) indicated that formation of the masculinized external genitalia of female spotted hyenas involves more than early androgen exposure and, therefore, can not be merely a secondary developmental consequence of selection for hormonally mediated traits. More probably, selection acted on an entire complex or cascade of developmental events (Arnold 1996; Ketterson & Nolan 1999). Although the present results do not permit discrimination between the various theories, they do emphasize the exceptional reproductive disadvantages of the high concentrations of androgens that circulate during foetal life in female spotted hyenas. Our study therefore calls for further attention to a search for benefits of androgenization that could offset such costs (Glickman *et al.* 1993).

Whereas the morphological changes resulting from prenatal anti-androgen exposure improve reproductive success in females, they appear to have the opposite effect in males. Our finding that no experimental male ever reproduced despite nearly eight years of cohabitation with females leads us to speculate that male spotted hyenas are physically incapable of overcoming the mechanical challenges of copulation when equipped with a 'feminized' penis. As previously proposed for other male mammals (Patterson & Thaler 1982), we suggest that modest changes in genitalic morphology lead to reproductive dysfunction in male hyenas.

Male spotted hyenas normally have a long penis, with a relatively complex distal morphology. The glans penis is covered with small spines and assumes an angular contour with a pointed tip (Frank *et al.* 1990). In numerous mammalian taxa, bacular (and hence penile) length is positively correlated with vaginal length (Patterson & Thaler 1982). Moreover, penile elongation is seen in primate species in which the females display sex skin: temporary genital swellings effectively increase the length of the females' reproductive tract (Dixson 1987). Based on such genitalic correspondence between the sexes, it seems likely that the distance imposed by the anterior location of the female spotted hyena's introitus (Drea *et al.* 1999) should be accompanied by a complementary degree of penile elongation.

Likewise, ornate penile morphologies appear to have evolved in conjunction with complex copulatory patterns, including genital locks, multiple intromissions and pelvic thrusting (Dewsbury 1975). For instance, in some primates, keratinized penile spines purportedly serve a mechanical function by gripping the walls of the female's reproductive opening during copulation (Dixson 1987). Moreover, the complex shape at the distal end of the baculum might assist males of many species to gain entry and/or support prolonged intromission (Long & Frank 1968; Patterson & Thaler 1982; Dixson 1987). Although copulation in spotted hyenas involves multiple, prolonged intromissions, undoubtedly placing significant pressure on the penis, members of Hyaenidae do not possess a baculum (Drea *et al.* 1999). Thus, in the absence of a baculum, the 'hooked' shape and surface texture of the glans penis may function to facilitate penetration and maintain intro-

mission. If the various components of penile morphology relate to reproductive function, modification of such features through prenatal anti-androgen exposure could negatively impact on male reproductive success. In summary, a shorter, thicker phallus with a rounded rather than angular contour is ineffectual for achieving entry, thereby rendering a male functionally infertile.

Our data support the hypothesis that a normal female hyena's exposure to endogenous androgens during foetal life modifies her penile clitoris in a manner that, at a much later date, exacerbates birthing difficulties during her first parturition. Thus, androgens or the epigenetic transmission of virilization in female spotted hyenas (Yalcinkaya *et al.* 1993) render an extremely unusual and difficult process even more reproductively costly. By contrast, a male's exposure to endogenous androgens during foetal life modifies his penis, giving him the length and contour necessary to mate with a highly masculinized female.

We thank the Office of the President, the Department of Wildlife Conservation and Management and the Narok County Council, Republic of Kenya, for permission to export infant hyenas to the Berkeley facility. We also extend our thanks to L. G. Frank for his pivotal role in initiating this line of research and establishing the captive hyena colony and to the staff of the Office of Laboratory Animal Care on the Berkeley Campus. Supported by NIH grants MH39917 and HD07684.

REFERENCES

- Arnold, A. P. 1996 Genetically triggered sexual differentiation of brain and behavior. *Horm. Behav.* **30**, 495–505.
- Cunha, G. R., Wang, Y., Place, N., Liu, W., Baskin, L. & Glickman, S. E. 2003 The urogenital system of the female spotted hyena (*Crocuta crocuta*): a functional histological study. *J. Morphol.* (In the press.)
- Davis, D. D. & Story, H. E. 1949 The female external genitalia of the spotted hyena. *Fieldiana Zool. (Chicago)* **31**, 277–283.
- Dewsbury, D. A. 1975 Diversity and adaptation in rodent copulatory behavior. *Science* **190**, 947–954.
- Dixson, A. 1987 Observations on the evolution of the genitalia and copulatory behaviour in male primates. *J. Zool. Lond.* **213**, 423–443.
- Drea, C. M., Weldele, M. L., Forger, N. G., Coscia, E. M., Frank, L. G., Licht, P. & Glickman, S. E. 1998 Androgens and masculinization of genitalia in the spotted hyena (*Crocuta crocuta*). 2. Effects of prenatal anti-androgens. *J. Reprod. Fertil.* **113**, 117–127.
- Drea, C. M., Coscia, E. M. & Glickman, S. E. 1999 Hyenas. In *Encyclopaedia of reproduction*, vol. 2 (ed. E. Knobil, J. Neill & P. Licht), pp. 718–725. San Diego, CA: Academic.
- East, M. L., Hofer, H. & Wickler, W. 1993 The erect 'penis' is a flag of submission in a female-dominated society: greetings in Serengeti spotted hyenas. *Behav. Ecol. Sociobiol.* **33**, 355–370.
- Fenstemaker, S. B., Zup, S. L., Frank, L. G., Glickman, S. E. & Forger, N. G. 1999 A sex difference in the hypothalamus of the spotted hyena. *Nature Neurosci.* **2**, 943–945.
- Frank, L. G. 1997 Evolution of genital masculinization: why do female hyenas have such a large 'penis'? *Trends Ecol. Evol.* **12**, 58–62.
- Frank, L. G. & Glickman, S. E. 1994 Giving birth through a penile clitoris: parturition and dystocia in the spotted hyena (*Crocuta crocuta*). *J. Zool. Lond.* **234**, 659–665.
- Frank, L. G., Glickman, S. E. & Powch, I. 1990 Sexual dimorphism in the spotted hyena (*Crocuta crocuta*). *J. Zool. Lond.* **221**, 308–313.

- Frank, L. G., Weldele, M. L. & Glickman, S. E. 1995 Masculinization costs in hyenas. *Nature* **377**, 584–585.
- Glickman, S. E., Frank, L. G., Davidson, J. M., Smith, E. R. & Siiteri, P. K. 1987 Androstenedione may organize or activate sex-reversed traits in female spotted hyenas. *Proc. Natl Acad. Sci. USA* **843**, 3444–3447.
- Glickman, S. E., Frank, L. G., Pavgi, S. & Licht, P. 1992 Hormonal correlates of 'masculinization' in female spotted hyenas (*Crocuta crocuta*). 1. Infancy to sexual maturity. *J. Reprod. Fertil.* **95**, 451–462.
- Glickman, S. E., Frank, L. G., Holekamp, K. E., Smale, L. & Licht, P. 1993 Costs and benefits of 'androgenization' in the female spotted hyena: the natural selection of physiological mechanisms. In *Perspectives in ethology*, vol. 10: behavior and evolution (ed. P. P. G. Bateson, P. H. Klopfer & N. S. Thompson), pp. 87–134. New York: Plenum.
- Glickman, S. E., Coscia, E. M., Frank, L. G., Licht, P., Weldele, M. L. & Drea, C. M. 1998 Androgens and masculinization of genitalia in the spotted hyena (*Crocuta crocuta*). 3. Effects of juvenile gonadectomy. *J. Reprod. Fertil.* **113**, 129–135.
- Gould, S. J. 1981 Hyena myths and realities. *Nat. Hist.* **90**, 16–24.
- Gould, S. J. & Vrba, E. S. 1982 Exaptation: a missing term in the science of form. *Palaeobiology* **8**, 4–15.
- Hamilton, W. J., Tilson, R. L. & Frank, L. G. 1986 Sexual monomorphism in spotted hyenas, *Crocuta crocuta*. *Ethology* **71**, 63–73.
- Herman, R. A., Jones, B., Mann, D. R. & Wallen, K. 2000 Timing of prenatal androgen exposure: anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Hormone Behav.* **38**, 52–66.
- Imperato-McGinley, J., Sanchez, R. S., Spencer, J. R., Yee, B. & Vaughan, E. D. 1992 Comparison of the effects of the 5 α -reductase inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: dose-response studies. *Endocrinology* **131**, 1149–1156.
- Ketterson, E. D. & Nolan Jr, V. 1999 Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* **154**, S4–S25.
- Kruuk, H. 1972 *The spotted hyena: a study of predation and social behavior*. University of Chicago Press.
- Licht, P., Zucker, I., Hubbard, G. & Boshes, M. 1982 Circannual rhythms of plasma testosterone and luteinizing hormone levels in golden-mantled ground squirrels (*Spermophilus lateralis*). *Biol. Reprod.* **27**, 411–418.
- Licht, P., Frank, L. G., Pavgi, S., Yalcinkaya, T., Siiteri, P. K. & Glickman, S. E. 1992 Hormonal correlates of 'masculinization' in female spotted hyenas (*Crocuta crocuta*). 2. Maternal and fetal steroids. *J. Reprod. Fertil.* **95**, 463–474.
- Licht, P., Hayes, T., Tsai, P.-S., Cunha, G. R., Hayward, S., Martin, M., Jaffe, R., Golbus, M., Kim, H. S. & Glickman, S. E. 1998 Androgens and masculinization of genitalia in the spotted hyena (*Crocuta crocuta*). 1. Urogenital morphology and placental androgen production during fetal life. *J. Reprod. Fertil.* **113**, 105–116.
- Long, C. A. & Frank, T. 1968 Morphometric function and variation in the baculum, with comments on the correlation of parts. *J. Mammal.* **49**, 32–43.
- Matthews, L. H. 1939 Reproduction in the spotted hyena *Crocuta crocuta* (Erleben). *Phil. Trans. R. Soc. Lond. B* **230**, 1–78.
- Muller, M. N. & Wrangham, R. 2002 Sexual mimicry in hyenas. *Q. Rev. Biol.* **77**, 3–16.
- Neaves, W. B., Griffin, J. E. & Wilson, J. D. 1980 Sexual dimorphism of the phallus in spotted hyena (*Crocuta crocuta*). *J. Reprod. Fertil.* **59**, 509–513.
- Neri, R. O. 1977 Studies on the biology and mechanism of action of nonsteroidal antiandrogens. In *Androgens and antiandrogens* (ed. L. Martini & M. Motta), pp. 179–189. New York: Raven.
- Patterson, B. D. & Thaeler Jr, C. S. 1982 The mammalian baculum: hypotheses on the nature of bacular variability. *J. Mammal.* **63**, 1–15.
- Racey, P. A. & Skinner, J. D. 1979 Endocrine aspects of sexual mimicry in spotted hyenas *Crocuta crocuta*. *J. Zool. Lond.* **187**, 315–326.
- Schneider, K. M. 1952 Einige Bilder zur Paarung der Flech-enhyäne, *Crocuta crocuta* Erxl. *Zoolog. Garten n.f.* **19**, 135–149.
- Siiteri, P. K. & Wilson, J. D. 1974 Testosterone formation and metabolism during male sexual differentiation in the human embryo. *J. Clin. Endocrinol. Metab.* **38**, 113–125.
- Thornton, J. E., Irving, S. & Goy, R. W. 1991 Effects of prenatal antiandrogen treatment on masculinization and defeminization of guinea pigs. *Physiol. Behav.* **50**, 471–475.
- Watson, M. 1877 On the female generative organs of *Hyena crocuta*. *Proc. Zool. Soc. Lond.* **24**, 369–379.
- Wilson, J. D., George, F. W. & Griffin, J. E. 1981 The hormonal control of sexual development. *Science* **211**, 1278–1284.
- Yalcinkaya, T. M., Siiteri, P. K., Vigne, J.-L., Licht, P., Pavgi, S., Frank, L. G. & Glickman, S. E. 1993 A mechanism for virilization of female spotted hyenas in utero. *Science* **260**, 1929–1931.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.