VAN JAARSVELD A S

ENDOCRINE CORRELATES OF SOCIAL DEVELOPMENT IN

THE SPOTTED HYAENA

CROCUTA CROCUTA (ERXLEBEN)

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Endocrine correlates of social development in the spotted hyaena

Crocuta crocuta (Erxleben)

by

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Dedicated to Santa, Barry and Nina

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Abstract

Patterns of hormonal secretion in spotted hyaenas with established social histories were monitored to establish how the endocrinology of the species is influenced by social development. A comparative study of testosterone, androstenedione, progesterone, oestradiol-17ß, and the stress related hormones, cortisol and prolactin was conducted after the establishment of suitable hormone assays.

An evaluation of hormonal 'provocation tests' as an index of androgen status was carried out, and revealed that there was a positive correlation between initial samples and LH-RH stimulation under the particular immobilisation and anaesthetic regime used. This enabled the direct comparison of individuals using the initial sample as an index of androgen status. Plasma testosterone and androstenedione concentrations reflected

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social development in males, while androstenedione showed elevated concentrations in neonatal females. This explains the inability of earlier studies to distinguish between the sexes using circulating androgens. Female androgen concentrations were largely determined by ovarian cyclicity and may be influenced by changes in plasma binding ability.

It was not possible to distinguish between the various female reproductive categories using plasma progesterone or oestradiol-17ß concentrations. However, the large variances of these hormones among the lactating females suggest that this species does not have a distinct lactational anoestrus.

Adreno-cortical and prolactin responsiveness to immobilisation stress supported some of the primate literature in that no distinct differences could be detected during dispersal and among various dominance related social categories. Moreover, the non-significant decline in basal cortisol concentrations in both male and female cubs suggests that the stress-hyporesponsive period is not as pronounced in this carnivore as in the rat. The reduced prolactin concentrations observed in lactating females are probably a function of an infrequent suckling pattern and may explain the lack of a lactational anoestrus in this species.

Multivariate analyses of differences in mean hormone levels identified the testsoterone levels in CIMs, the oestradiol-17ß levels in PFs and the prolactin concentrations in LFs as extreme endocrine conditions. An analysis of the nature of endocrine variance within a multidimensional hypervolume identified androgens in males, and the contrast between androstenedione and cortisol concentrations as the main hormonal variant in females.

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CHAPTER 1 - INTRODUCTION

The spotted hyaena

After suffering centuries of persecution, through the actions of our illustrious forbears, narrators and naturalists alike (Gould 1981), we can finally say that the spotted hyaena (*Crocuta crocuta* Erxleben) is coming into its own right. Its resurrection from a mythical past can be attributed largely to the classic work by Hans Kruuk (1966, 1972). His research contributed greatly to the spotted hyaena, the second largest extant mammalian carnivore in Africa, taking its rightful place alongside the other major predators. The revival of interest by the biological community in this species, is admirably demonstrated by the large number of recently completed and ongoing projects, aimed at investigating all aspects of *Crocuta* biology.

Kruuk's (1966, 1972) study revealed three important features of spotted hyaena socio-ecology. First, that spotted hyaenas are effective nocturnal predators in their own right, and not merely scavengers of kills made by other predators. Secondly, that they have a flexible social system which allows them to exploit the available resources effectively by changing from solitary to social hunting or *vice versa* at very short notice. Moreover, this flexibility allows them to establish territorial social groupings, called clans, in areas where prey is sedentary. The third major finding by Kruuk was that females are behaviourally dominant over males within such clans. Although many aspects of spotted hyaena biology have been

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investigated over the last decade (see Henschel 1986), the most significant advances have been made in elucidating the nature of such a spotted hyaena social unit or clan.

Social organisation

It is now well established that the nucleus of the spotted hyaena clan is a variable number of closely related matrilocal lineages (Mills 1985, Frank 1986a, Henschel & Skinner 1987). Associated with this core of closely related females is a multi-male group comprising males in various stages of immigration or emigration (Frank 1986a, Henschel & Skinner 1987). Studies in both East - and South Africa, found that the dispersal pattern differs between the sexes. Females are generally philopatric and remain in their natal clans to breed. Males on the other hand tend to disperse around puberty and join neighbouring clans, where they attempt to obtain mating opportunities (Frank 1986a, Henschel & Skinner 1987). This emigration is not without risk, as the acceptance of a male into a new clan may take up to several months, during which time agonistic encounters with members of the target clan are often experienced (Henschel 1986, Henschel & Skinner 1987). That it is this sex-biased dispersal strategy which is responsible for a higher male mortality rate in spotted hyaenas also seems conclusive (Henschel 1986, Frank 1986a). This sex-biased dispersal strategy, together with the observed female philopatry indicates that the spotted hyaena clan can be classified as a female-bonded group (Wrangham 1980).

Besides elucidating aspects related to spotted hyaena social organisation, both the South - and East African studies confirmed Kruuk's (1972) finding that females are generally behaviourally dominant over males. Although

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earlier reports had suggested that this female dominance may be partly due to their being larger than males (Matthews 1939, Deane 1962, Wilson 1968, Kruuk 1972, Racey & Skinner 1979), the current concensus of opinion is that female spotted hyaenas are not necessarily larger framed than their male counterparts, and that their larger bulk is a result of their relative dominance over males. This dominance gives them priority access to food and means that a larger mass is a result rather than the cause of dominance (Hamilton, Tilson & Frank 1986, Henschel & Skinner 1987, Van Jaarsveld, Skinner & Lindeque 1988).

This alternative view can be ascribed to the findings that dominance in this species could not be linked to simple developmental criteria such as age, body size or reproductive activity (Frank 1986b, Henschel & Skinner 1987), and that the cultural transmission of relative rank played an important role in spotted hyaena social relationships. This inheritance by offspring of "dependent rank" (Kawai 1958) from their mothers, where the cubs' rank is related to that of the mother, together with the sex-biased dispersal strategy both contribute towards the emergence of female dominance and the low ranks of mating males. Such acquired rank can only be expressed within the social context in which it was inherited, and as males usually leave their natal clans to join others, they forfeit this dependent rank in order to achieve matings elsewhere (Henschel 1986). The lack of male coalitions in this species means that any immigrant male is at a competitive disadvantage against the resident philopatric females of a target clan. Consequently, despite being responsible for most of the matings (Kruuk 1972, Frank 1986b, Henschel & Skinner 1987), immigrant males are near the bottom of the social hierarchy.

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Dominance relationships within a spotted hyaena clan seem to be very stable (Henschel 1986, Frank 1986b, Henschel & Skinner 1987). Henschel & Skinner (1987) found a near linear hierarchy in a small clan comprising 14 individuals, whereas Frank (1986b) could only discern linear dominance relations for some females in a much larger clan comprising 62-80 individuals. Despite difficulties in obtaining sufficient interactions from lower ranking animals, Frank (1986b) nevertheless concluded, on the strength of occasional observations, that there are stable dominance relations among lower ranking animals. However, Frank's (1986b) analysis compared females to males as two separate classes. An alternative and more informative approach was adopted by Henschel & Skinner (1987), who used the dispersal pattern of the species as a guideline for the delineation of social classes. This resulted in the distinction between the following social groups: females; cubs; and three social classes of males, namely, resident natal males (RNM); peripheral immigrant males (PIM) and central immigrant males (CIM). A tenet of sociobiology is that any social system, such as the spotted hyaena clan, represents a history of social interactions between individuals and may be interpreted as the cumulative result of individual dispersal tactics. Consequently, the way that members of a spotted hyaena clan maintain or gain their status may be described in terms of the retention of, or transfer between any one of the following five dispersal stages (Henschel & Skinner 1987):

(i) dependent cubs (< 2 years), which are non-breeders in their natal clans;

(ii) permanently resident females, which have become reproductively active;

(iii) resident natal males (RNM), which were born into the clan and have not yet dispersed;

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(v)   peripheral immigrant males (PIM), which are dispersing males and associate with the clan temporarily

(iv)   central immigrant males (CIM), which became residents and are responsible for most of the matings (see also Frank 1986a);

Moreover, the above authors found that mature females were the dominant social group, followed by other natal members, CIMs and PIMs in that order. Their work therefore indicated that these various dispersal stages in actual fact represent a continuum of both social and reproductive developmental stages, and that this broad classification might prove useful when trying to describe either the social or reproductive development of this species. For this reason, the pattern of socio-endocrine development in this species was investigated in terms of these well defined and relatively easily discernable dispersal stages (see Henschel 1986).

Reproductive patterns

Spotted hyaena reproduction has been a bone of contention for a number of years. The reasons for this controversy are twofold: first, the lack of a distinct genital dimorphism in this species (Watson 1877, Matthews 1939, Kruuk 1972, Racey & Skinner 1979, Neaves, Griffin & Wilson 1980, Gould & Vrba 1982, Lindeque & Skinner 1982a, Hamilton, Tilson & Frank 1986, Van Jaarsveld & Skinner 1987) and the more recently adressed issue concerning a possible endocrine basis for female dominance in this species (Racey & Skinner 1979, Frank, Davidson & Smith 1985a, Frank, Smith & Davidson 1985b, Hamilton *et al.* 1986, Glickman, Frank, Davidson, Smith & Siiteri

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1987, Lindeque, Skinner & Millar 1986, Van Jaarsveld & Skinner 1987). In view of the rapid developments in this field, a short review explaining current understanding of *Crocuta* reproductive patterns follows.

The spotted hyaena is known for its aseasonal breeding pattern throughout its present range. In addition, evidence that this aseasonality is characterised by the presence of one or more birth peaks is accumulating (Kruuk 1972, Lindeque & Skinner 1982b, Frank 1986a). In the male, testicular histology was found to conform to the general mammalian pattern. Male spermatogenic activity is evident at approximately two years of age (Matthews 1939, Lindeque 1981), and although age related increases in testes mass, epididymal mass and epididymal sperm reserves have been reported (Lindeque 1981), it is uncertain how any of these parameters may affect male fertility. The most significant observation regarding male reproduction is that although there is no apparent seasonality, a significant proportion of males in the reproductively active age groups seem incapable of breeding at any time (Matthews 1939, Lindeque 1981).

On the other hand reproductive development in females takes somewhat longer than males, and they only reach maturity at approximately three years of age (Schneider 1926, Matthews 1939, Kruuk 1972, Lindeque 1981, Frank 1986a). Furthermore, it is well established that *Crocuta* is polyoestrus. Behavioural observations have indicated that the oestrous cycle is approximately 14 days long (Grimpe 1916, Schneider 1926), and intermittent anoestrous periods ranging from 14 - 60 days have been reported (Grimpe 1916, Schneider 1926, Golding 1969). The luteal phase lasts longer than half of the oestrous cycle, and corpora lutea are persistent (Matthews 1939, Lindeque 1981). Pregnancy lasts for approximately 110 days (Grimpe

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1916, Schneider 1926), and recurring oestrous cycles are responsible for the presence of the multiple generations of corpora lutea visible in the ovaries of adult females (Lindeque 1981). The duration of lactation seems to be highly variable (Matthews 1939, Kruuk 1972), and although lactational anoestrus seems to be the norm, some degree of follicular development and even pregnant lactating females have been reported (Lindeque 1981). The established endocrine secretion patterns in this species will be discussed in the respective chapters aimed at further clarification of those issues.

Development

One of the principal aims of the present thesis is to investigate the patterns of endocrine secretion within the spotted hyaena socio-ecological context. Socio-ecological development is, in turn, governed by reciprocal stimulative processes between animals and their respective environments, and is therefore extensively influenced by social phenomena. Moreover, communication between an animals' internal and external environment is facilitated through two coordinating systems, namely the nervous system and the endocrine system. This communication process leads to responses that intimately reflect the effect of environmental stimulation, and results in potentially adaptive physiological and behavioural responses. However, organic development is the result of both maturative and experiental influences; maturation involves the contributions of tissue growth and differentiation, and experience involves the effects of environmental stimulation and its organic correlates on behaviour (Illius 1976). Although the experiential influences of development form the central thrust of this dissertation, there is little doubt that the maturative component makes an important contribution towards the total socio-ecological development of an

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organism. This explains the relatively large body of literature aimed at investigating these maturative aspects of spotted hyaena development. Questions adressed to date include: birth and early development (Pournelle 1965, Golding 1969, Henschel 1986), litter sizes (Frank 1986b, Van Jaarsveld *et al.* 1988), the origins of sexual monomorphism (Kruuk 1972, Racey & Skinner 1979, Gould & Vrba 1982, Hamilton *et al.* 1986, Glickman *et al.* 1987, Van Jaarsveld & Skinner 1987), behavioural differentiation (Glickman *et al.* 1987) and species specific growth (Van Jaarsveld *et al.* 1988).

In contrast, with the intensive research programme aimed at clarifying these maturative processes the contribution of experiential influences have to date been neglected. That experiential development occurs in the spotted hyaena in response to environmental stimulation, and that it is associated with changes in behaviour is well established (Frank 1986b, Henschel & Skinner 1987). However, the identification of the organic correlates of this reciprocal stimulative process remains relatively obscure. In this regard the various dispersal stages identified earlier (Henschel & Skinner 1987) may prove informative. Although this project only makes a limited contribution to experiential effects of the pre-weaning stages, socio-ecological development is continuous throughout the duration of an animals lifespan, and these various dispersal stages form a convenient departure point for the identification of organic correlates that reflect spotted hyaena socio-ecological development.

Of the two systems involved in this two-way communication between an organism and its environment (Illius 1976): the nervous system is mainly responsible for the development of appropriate conditioned reflexes and

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autonomic emergency reactions, whereas the endocrine system is associated with raising the non-specific resistance of an organism to biological stressors. This latter mechanism allows an organism to adapt its physiology or internal environment to the contraints imposed on it by the external environment (Selye 1971). This non-specific and pliable nature of the endocrine system when compared to the nervous system makes it a more likely candidate for revealing organic correlats of behavioural development. Consequently, a synopsis of the interaction between behaviour on the one hand and endocrine secretory patterns on the other, will serve to introduce the adopted approach.

Social stress

Animals appear to possess a complex hormonal defensive system comparable in its scope to those based upon nervous or immunological reactions. This hormonal defensive system, originally termed the *General Adaptation Syndrome* (Selye 1971), is reportedly activated by numerous stressors such as surgery (Matsumoto, Takeyasa, Mizutani, Hamanaka & Uozumi 1970), immobilisation (Sapolsky 1982), foot shock (Selye 1971) and prolonged psychological stress (Kreuz, Rose & Jennings 1972). Furthermore, incidental observations have indicated that resistance to many pathogens can be directly enhanced or diminished by an excess or deficiency of thyroid, adrenal, gonadal and pancreatic hormones. These may be secreted in response to a need, or they may modify reactivity merely through their continuous presence in the body. These hormones all increase resistance, though not necessarily against the same agents or through the same mechanisms (Selye 1971). Over the last decade it has also become apparent that behavioural subordinance constitutes just such a stressful condition for most animals

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(see Abbott 1987 for review). This led to the realisation that stress may not necessarily be a pathological response in the experiental development of animals, and that any non-specific deviation from a resting state which stimulates repair, may in fact be referred to as a stressor. Consequently, stress may be a natural and inevitable part of life which need not be overdramatised (Ricklan & Levitan 1969).

The central issue here is whether this plethora of endocrine mechanisms, originally referred to as the General Adaptation Syndrome, play any significant role in directing the effect that social experience may have on the social development of animals. Numerous studies have indicated that this may be the case (see Abbott 1987), and the most significant correlations between relative social status and hormonal parameter estimates have been found in the hypothalamo-pituitary-adrenal axis (Selye 1971, Keverne 1979, Baxter & Tyrell 1987) and the hypothalamo-pituitary-gonadal axis (Gordon, Rose & Bernstein 1976, Keverne 1979, Sapolsky 1982, Abbott 1987). As most of these results were obtained through social manipulation studies, it is not yet conclusively established to what extent these hormonal defensive systems may be involved in directing social development in natural unmanipulated social groups. Positive results have been obtained in a study on wild male olive baboons (*Papio anubis*) - (Sapolsky 1982, 1983a), however, this study of the endocrine correlates of progressive and natural social development in the spotted hyaena is the first investigation to focus on all the elements of the established social structure, and the first on a social carnivore.

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CHAPTER 2 - MATERIAL AND METHODS

Study animals

A number of projects related to all aspects of *Crocuta* biology presently in progress in Southern Africa were the sources of most of the material used in this study. Thirty spotted hyaenas (> 1 yr) with established social histories were sampled between July 1984 and July 1987 from the following clans:

    (i) the Mavumbye clan which is resident in the Central District of the Kruger National Park (24^O20'S/31^O45'E) - (see Henschel 1986, Henschel & Skinner 1987),

    (ii) the Shingkelengane clan's home range is in the Central District of the Kruger National Park (24^O20'S/31^O46'E) and the Letaba clan that is resident around the Letaba Rest Camp in the Kruger National Park (23^O56'S/31^O40'E) were observed during the present study.   (iii) the Kousaunt clan's home range straddles the boundary between the Kalahari Gemsbok National Park (South Africa) and the Gemsbok National Park (Botswana) - (25^O15'S/20^O30'E). This clan is the subject of a long-term behavioural ecology project (see Mills 1985, Knight, Van Jaarsveld & Mills submitted).

    (v)   the National Zoological Gardens (Pretoria) breeding colony.

The protocols used to determine the social histories of all individuals were the same in all of the above studies. Parameters used to evaluate dispersal categories were as defined by Henschel (1986) and Henschel & Skinner (1987).

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In addition, 12 cubs ($\leq$ 1 yr) were sampled from various localities in the Kruger National Park as well as from the National Zoological Gardens (Pretoria). This was an effective way to increase the sample size for this younger age group, as they do not disperse before attaining reproductive maturity (see Kruuk 1972, Frank 1983, Mills 1985, Henschel 1986), and consequently the social histories of spotted hyaena cubs may readily be inferred. Animals were subsequently classified as belonging to one of the following social categories: female cubs ($\leq$ 1 yr), resident females (> 1 yr), male cubs ($\leq$ 1 yr), resident natal males (RNM), peripheral immigrant males (PIM) or central immigrant males (CIM).

Immobilisation

All animals were immobilised with Zoletil (CI-744: Anchorpharm Pty. (Ltd) Bramley, S.A.) following Van Jaarsveld (1988). Extended anaesthesia (4h) for serial blood sampling was achieved by administration of halothane (Fluothane: I.C.I. Pharmaceuticals Ltd, Johannesburg, S.A.) using a circle absorber machine. Induction was achieved with a 10% mixture of halothane and oxygen and the animals were maintained using 2 - 4% halothane in a closed circuit. This method was preferred as it provided a more stable plane of anaesthesia than other chemical immobilising agents. Clinical procedures followed Van Jaarsveld, McKenzie & Meltzer (1984).

Blood sampling procedures

Adults were subjected to either serial blood sampling or once-off blood collecting events. Serial sampling from cubs was not feasible and they were thus subjected to once-off sampling per immobilisation. All plasma

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(heparinised - 10 ml) and serum samples (10 ml) were collected from the cephalic or saphenous veins using multi-sample needles and venoject evacuated tubes. Samples were stored upright at $4^{O}C$ until centrifuged for 10 min at 3000 rpm. Plasma and serum were stored at $-20^{O}C$ until assayed.

Serial sampling: Serial sampling in the field was started as soon as possible following immobilisation. Animals were left to stabilise for 90 min following immobilisation, during which time they were weighed, intubated and attached to a circle absorber machine. A Ringer-lactate drip (Keagrams Ltd, Johannesburg, S.A.) was inserted in the *vena jugularis* to replace fluids lost due to blood sampling ($\pm$ 300 ml) and to maintain a constant blood pH during prolonged anaesthesia. After 90 minutes the animals were administerd 1 ug LH-RH (Hoechst, Frankfurt, West Germany) per kg body weight intravenously (Illius *et al.* 1983). Mass dependent doses were administered to compensate for peripheral dilution in animals of different ages and body sizes. Serial blood samples were collected at 15 min intervals for the total duration of anaesthesia (4h). Also, body temperature was monitored intermitently and adjusted using hot water bottles and solar blankets.

Age estimation

Most of the Kruger National Park animals were of an unknown age and were subsequently aged following Van Jaarsveld, Henschel & Skinner (1987). In order to improve accuracy both third mandibular premolar (PM_3) height and surface area were used. Known age animals such as the Kalahari (Mills 1985, Knight, Van Jaarsveld & Mills submitted) and Zoo animals were recorded as such.

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Reproductive status

Reproductive status was assessed through external examination according to the criteria specified by Matthews (1939) and Lindeque (1981). After age estimation animals were finally classified as belonging to one of the following reproductive categories: nulliparous female, parous female, lactating female, immature male ($\leq$ 2 yrs), and mature male (> 2 yrs).

Statistics and Laboratory procedures

All statistical procedures used were in accordance with the statistical principles described by Sokal & Rohlf (1980). Statistical manipulations were carried out on the University based mainframe computer using SAS Institute Inc. (Illinois, USA) software. The relevant procedures used are specified together with the results. The frequency distributions of all data sets were tested for normality, and variances for homoscedasticity. Where raw data failed to comply with the required criteria for parametric analyses, data transformations were employed in order to obtain normality and/or homoscedasticity. In instances where data sets or subsets still failed to comply with parametric criteria non-parametric statistical procedures were utilised.

Laboratory procedures for the Radiochemical determination (RIA) of hormone concentrations were carried out in accordance with the reliability criteria described by Jeffcoate (1981).

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Sample characteristics

The very nature of this project, aimed at investigating developmental aspects of sociality, will inevitably lead to several analyses of age related phenomena. This requires a careful appraisal of the statistical nature of the study sample, especially its distribution characteristics. Failure to comply with parametric criteria would severely hamper effective statistical treatment and conclusive analyses. The raw age-related data were not normally distributed, however a simple $\log_{10}$ transformation of this distribution is depicted in Fig. 1.
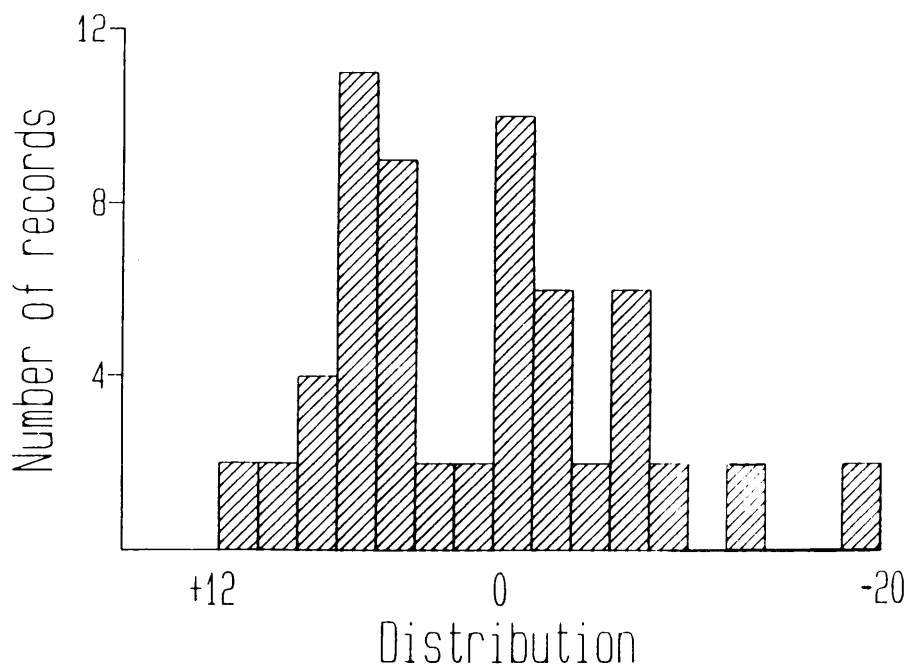
Fig.1. The frequency distribution for the study sample depicting the median age ($\log_{10}$) age.

The inability of the $\log_{10}$ age data to comply with normality can be attributed to the lack of samples in the 1-2 year age category. The rest of

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the distribution seems to form a very normal distribution pattern. As this lack of normality is apparently related to sampling error and not necessarily a population characteristic, the assumption of normality for age related analyses seems justified. Age related criteria will therefore be investigated using parametric methodology (Sokal & Rohlf 1980) when possible.

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CHAPTER 3 - ANDROGEN STATUS

Introduction

Androgens have featured extensively in the ongoing debate concerning the existance of endocrine correlates of relative dominance in the spotted hyaena. These investigations were primarily interested in elucidating the phenomenon of female dominance (Kruuk 1972, Frank 1986a, Frank 1986b, Henschel & Skinner 1987), and seemingly conflicting results have emerged. Studies that measured testosterone levels in southern African hyaenas found no significant difference between male and female testosterone titres (Racey & Skinner 1979, Lindeque *et al.* 1986), whereas samples collected in an East African population reportedly had higher testosterone levels in males (Glickman *et al.* 1987). Similarly, when total androgens were measured in the same East African population, females also had androgen levels significantly lower than males (Frank *et al.* 1985a). This latter study also concluded that the androgen levels in both sexes were strongly affected by individual social status, with the alpha animals in each sex having the highest androgen levels (Frank *et al.* 1985a).

The aims of this section of the project were two-fold: first, to confirm previously published findings concerning peripheral plasma androgen titres, using a pituitary challenge technique (Lindeque *et al.* 1986) as an estimate of androgen status. In addition, changes in the patterns of peripheral plasma androgens were related to developmental criteria such as age, as well as the various reproductive and social categories identified earlier (see pg. 4).

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Testosterone

Methods

<u>Testosterone assay</u>: Plasma testosterone was assayed using the same chemical reagents, and following identical procedures previously described by Van Aarde & Skinner (1986). Concentrations of peripheral plasma testosterone were estimated using duplicate plasma aliquants of 0,05 - 0,5 ml, and using antisera raised in rabbits against testosterone-3-carboxymethyl-oxime conjugated to bovine serum albumin (Millar & Kewley 1976).

<u>Testosterone status</u>: A number of parameter estimates were tested to determine whether any of them showed biases in testosterone status estimation. The tested parameter estimates were:

a) <u>Initial sample</u> - which was represented by the first blood sample collected from an immobilised hyaena, irrespective of the elapsed time after darting.

b) <u>Immobilisation response</u> - was represented by the relative area beneath the response curve obtained through the connection of measured serial testosterone titres collected during the first 90 min of anaesthesia (Fig. 2). The largest area measured with a graphic tablet and using a CADDIE (Letter Graphics, Johannesburg) graphics system, was assigned a value of one. In order to obtain a relative ranking all measured areas were then expressed as a relative fraction of one.

c) <u>LH-RH response</u> - the procedure used to estimate the extent of testosterone secretion in response to LH-RH stimulation was exactly the same as described for the immobilisation response. The relative response was, however, estimated using the measured serial

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testosterone titres collected from the time of LH-RH administration to the termination of anaesthesia: 90 - 240 min after immobilisation (Fig. 2).

Testosterone binding: Preliminary investigations aimed at establishing the extent of testosterone binding in spotted hyaena plasma were conducted using a charcoal absorption assay: samples from at least three individuals of those categories which presumably provide the highest resolution, namely reproductive categories for females and social categories for males, were analysed. To duplicate samples (200 μl) was added [1,2,6,7-^{3}H]testosterone (sp. act. 349 mCi/mg; Radiochemical Centre, Amersham, U.K.) in 0,1 ml phosphate buffer ($\sim$ 10 000 c.p.m.). After mixing, tubes were incubated at 37°C for 60 min and at 4°C for a further 30 min. Separation of plasma-bound and free testosterone was carried out at 4°C by addding 0,75 ml dextran coated charcoal consisting of a suspension of charcoal (Aktivole; Merck, Darmstadt, F.R.G.) in assay buffer (0,156 g/ml) containing 0,0156 g Dextran T-40 (Pharmacia, Uppsala, Sweden) to each tube. After mixing gently for 20 sec, tubes were incubated at 4°C for 10 min and centrifuged at the same temperature at 1500 g for 10 min. The supernatants were decanted into scintillation vials, 4 ml scintillation fluid (Scintillator 299^{TM}; Packard Instrument co., Illinois USA) was added, and measured 4 h later for 2 min, using a Packard 1500 Tri-Carb scintillation counter (Packard Instrument Co., Illinois, USA). Recovered counts were taken to represent plasma binding. Corroboration of this assumption was provided by subjecting serial dilutions (1:0, 1:1, 1:2, 1:4, 1:8. 1:16 in phosphate buffer) of all plasma samples to the same procedure (Fig. 5).

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Results

Testosterone assay validations: Antiserum specificity was described by Millar & Kewley (1976). Cross-reaction with all major steroids was < 0,1% except for dihydrotestosterone for which it was 5,1%. Assay sensitivity ranged from 40 to 290 pg/ml ($\bar{x}$ = 140,30 $\pm$ 96,81 s.d.; n = 12). Recovery estimates for a plasma pool ranged from 85 - 98% ($\bar{x}$ = 91,18; n = 12) for 100 ul samples and was 85% for 500 μl samples (n = 1). Intra- and inter-assay coefficients of variation were 7,8% and 9,8% respectively. Buffer blanks contained 12,17 $\pm$ 0,11 (n = 12) pg testosterone equiv./ml. Parallelism was demonstrated over the whole range of the standard curve by serial double dilution of plasma samples. Accuracy was estimated by the addition of 250, 500, 1000, 2000, 2500, 3750 and 5000 pg testosterone/ml (Δ^4-androsten-17-ol-3-one; Sigma Chemical Co., Dorset, U.K.) to a plasma pool, and resulted in recoveries of 105 $\pm$ 17% (n = 7) over the range.

Testosterone status: The obtained testosterone profiles for all animals investigated are given in Fig. 2. The most striking feature about these profiles is their inability to conform to expected theoretical response curves (Illius, Lamming, Howles, Fairall & Millar 1983). Not an unexpected result in the light of earlier demonstrations that both the adrenals and gonads contribute towards peripheral testosterone concentrations (Lindeque et al. 1986). All the investigated parameter estimates of testosterone status, namely, initial samples, after immobilisation and LH-RH administration revealed similar predictive values. This is apparent from the high rank correlation (r_s) between these different parameter estimates (Table 1). These data indicate that the accuracy of any of these measured parameter estimates in predicting relative testosterone status in this

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species do not necessarily differ. Consequently, the value of the initial sample was used in all further analyses of testosterone status. This both increased the effective sample size and allowed direct comparison between adult spotted hyaenas and younger individuals for which serial sampling was not feasible.
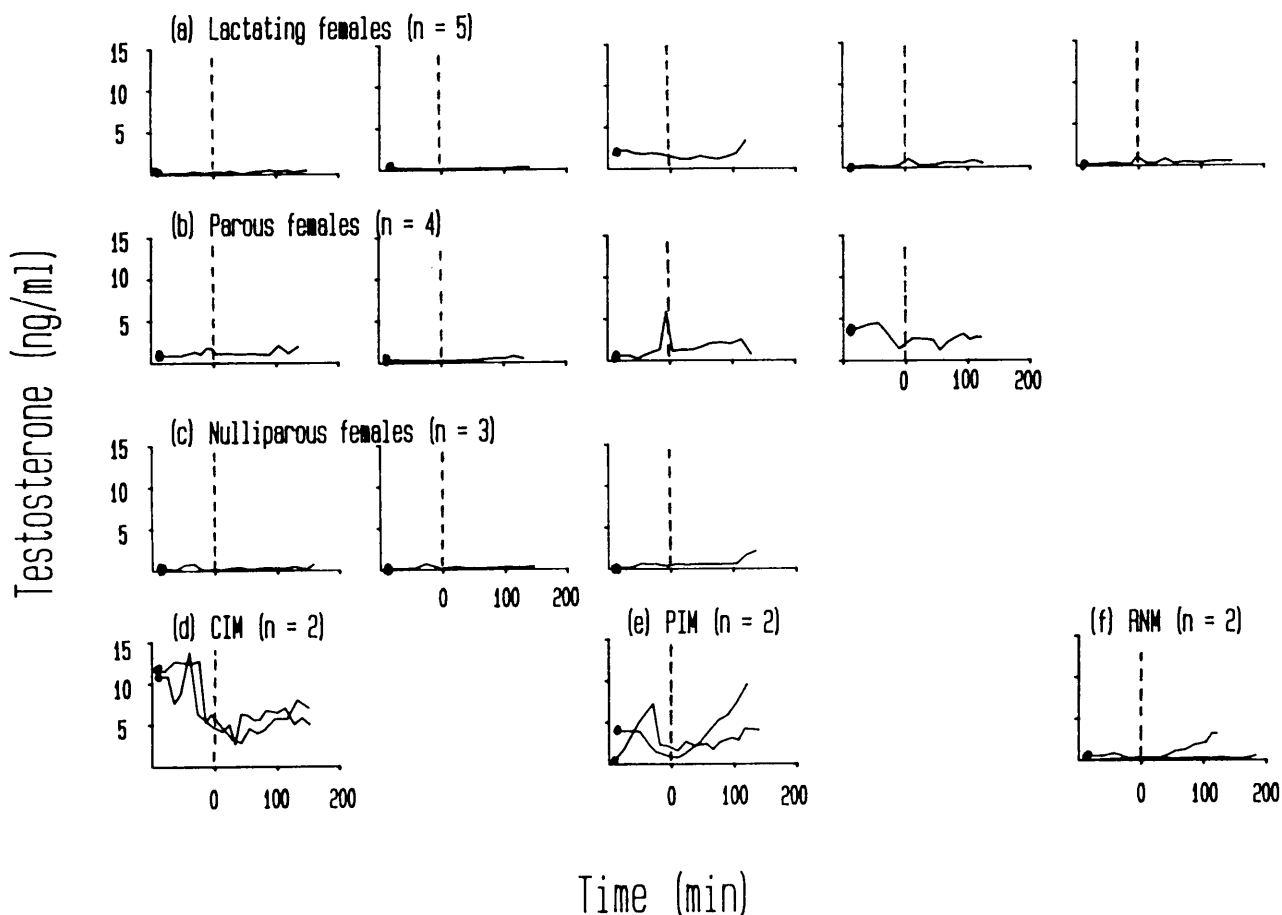
FIG. 2. Observed testosterone profiles for eighteen spotted hyaenas anaesthetised with Halothane following Zoletil immobilisation. The three testosterone status parameter estimates, namely, initial sample (●), immobilisation response (-90 to 0 min) and LH-RH response (0 to 150 min) are indicated. (a) Lactating females, (b) parous females, (c) nulliparous females, (d) central immigrant males, (e) peripheral immigrant males and (f) resident natal males.

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TABLE 1. Calculated relationships between three parameter estimates of testosterone status in the spotted hyaena (n = 18). All estimates were collected from the same animals during a single immobilisation. SAS PROC CORR SPEARMAN (r_s) was used.

Parameter estimates	Spearman's coefficient (r_s)	Significance level
1. Initial sample *vs* immobilisation response	0,82	P < 0,001
2. Initial sample *vs* LH-RH response	0,77	P < 0,001
3. Immobilisation response *vs* LH-RH response	0,91	P < 0,001

Age related analyses of testosterone status in this species resulted in a number of interesting trends. Although a positive rank correlation between age and peripheral testosterone titres of animals older than one year for the sample as a whole (PROC CORR - Pearson's correlation coefficient; r = 0,42; P < 0,05; n = 31) could be demonstrated, this was mainly due to the significant relationship of testosterone with age shown by males (r = 0,69; P < 0,01; n = 15). Females showed no increase in circulating testosterone with age (r = 0,23; P > 0,1; n = 17). This indicates that peripheral testosterone titres may reflect developmental processes in males and not in females.
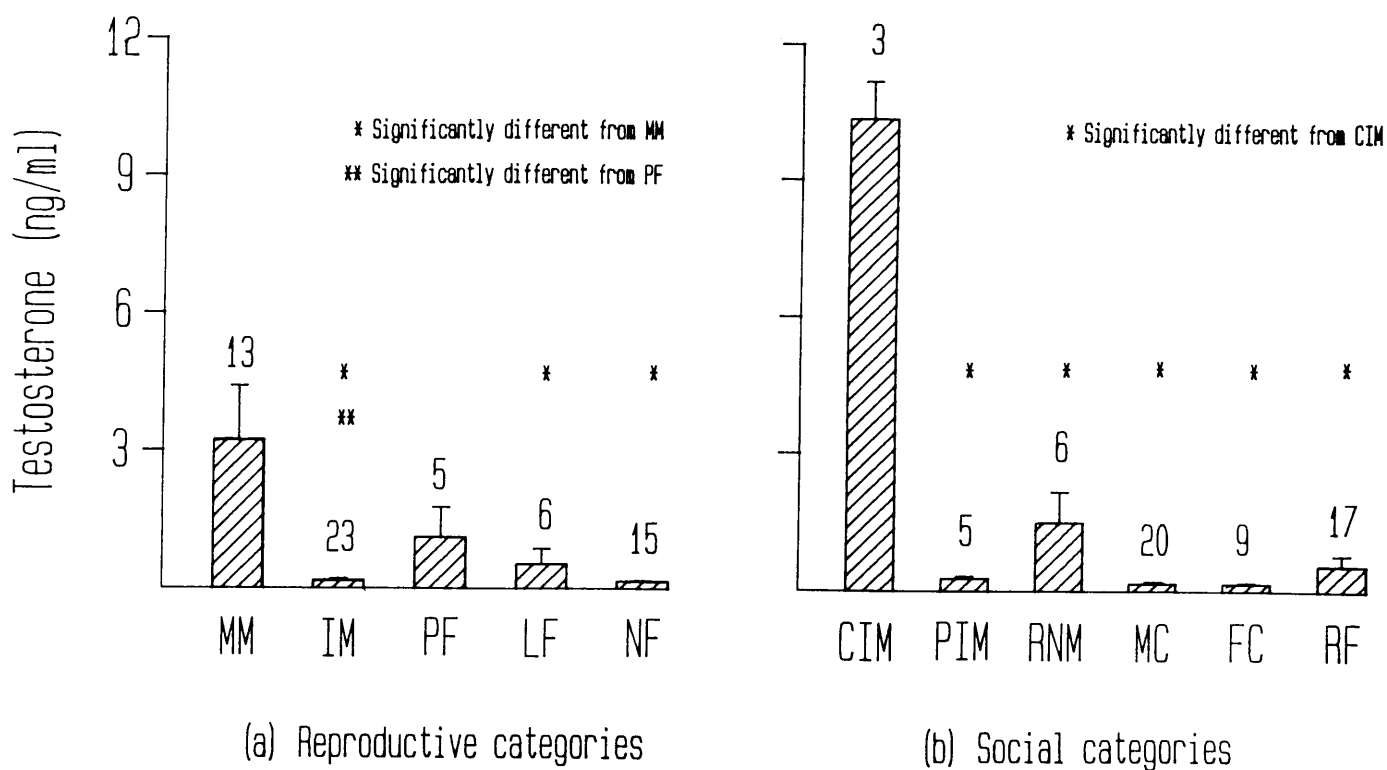
FIG. 3. Mean (± s.e.m.) peripheral testosterone titres for:

(a) Reproductive categories: MM = mature males (> 2 yrs), IM = immature males (≤ 2 yrs), PF = parous females, LF = lactating females, NF = nulliparous females, and

(b) Social categories: CIM = central immigrant males, PIM = peripheral immigrant males, RNM = resident natal males, MC = male cubs, FC = female cubs and RF = resident females. ANOVA was run on $\log_{10}$ testosterone. Statistical significance was measured at the level of P < 0,05.

Non parametric analysis of variance (PROC NPARIWAY - Kruskal-Wallis: Chi-square approximation; X_1 = 0,88; P > 0,05) revealed no significant differences in peripheral testosterone levels among the sexes. Parametric

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ANOVA (PROC GLM) comparing $\log_{10}$ testosterone levels for the different reproductive categories of spotted hyaenas (Fig. 3a) showed significant differences ($F_{0,05[4,57]} = 11,67$) between groups. Comparison of means (Tukey's studentized range - HSD) revealed that mature males (> 2 yrs) had significantly ($HSD_{57} = 3,984$; P < 0,05) higher mean testosterone levels than all other reproductive categories, except for parous females. Also, parous females had higher mean testosterone levels than immature males (Fig. 3a). A similar analysis of variance of $\log_{10}$ testosterone titres among respective social categories also showed significant variance ($F_{0,05[5,54]} = 9,01$). Central immigrant males (CIM) had significantly higher ($HSD_{54} = 4,178$; P < 0,05) mean testosterone levels than all the other social categories (Fig. 3b).

The observed testosterone titres found in several cubs of different ages are shown in Fig. 4. Although no correlation between age and peripheral testosterone titres was observed for cubs (r = 0,33; P > 0,05; n = 30) the male cub data set did show a significant inverse relationship (r = -0,46; P < 0,05; n = 20). On the other hand, visual analysis of the profiles obtained for both sexes, illustrate that the testosterone levels measured were consistently lower in male and female cubs than those observed in adult animals (see Fig. 3). The only exception, and the cause of the observed negative correlation for male cubs, was the single sample obtained from a four day old male (Fig. 4b) which approached concentrations titres recorded in some adult specimens. A notable feature of this young male animal was that the testes were descended at 4 four days of age, but had disappeared again by 18 days, only to reappear at an age of five months.

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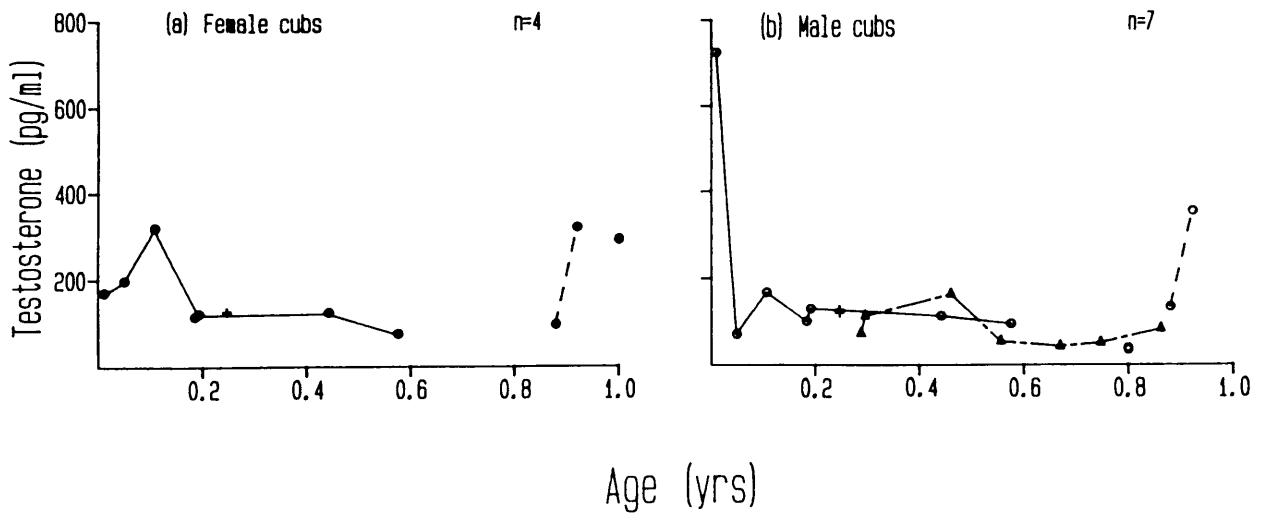
Fig. 4. Peripheral testosterone titres measured in 11 cubs. Samples collected from the same individuals are connected to one another.

Testosterone binding: The observed responses to serial dilution of plasma samples before the performance of a charcoal absorption assay indicate that ^{3}H-Testosterone is bound to proteins in spotted hyaena plasma. Differences in plasma testosterone binding capacity obtained in various categories of spotted hyaenas are illustrated in Fig. 5. Plasma testosterone binding seemed to vary little between the various categories, with the exception of those categories that represent adult females, namely parous and lactating females. These categories displayed a higher plasma testosterone binding in some individuals. The most significant feature of testosterone binding in these adult females is, however, the variation that is found within those categories (Fig. 5).

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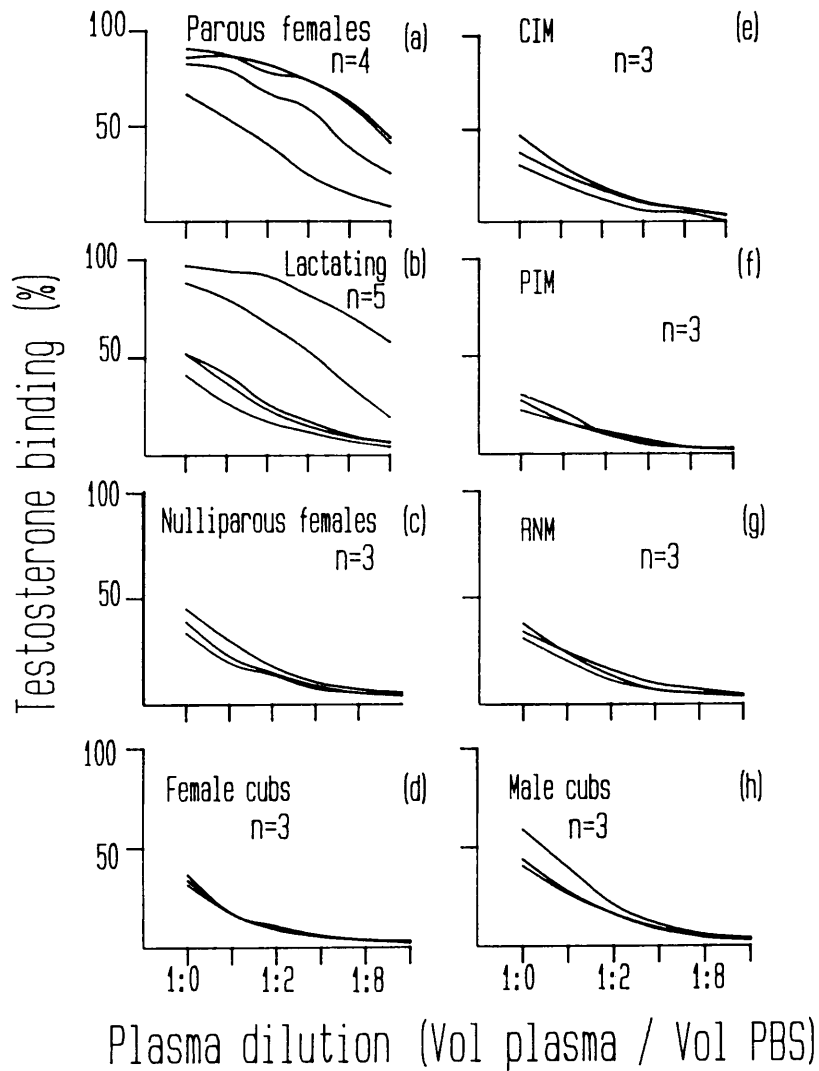
Fig. 5. Percentage testosterone binding in various categories of spotted hyaenas, showing the effect of serial plasma dilution on binding ability, the large variation in binding ability among adult females, as well as the higher testosterone binding ability of certain adult females when compared to all other categories.

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Androstenedione

Methods

Androstenedione assay: Plasma androstenedione was assayed by radio-immunoassay using duplicate plasma aliquants (0,1 ml) following extraction with 4 ml of a 4:1 (v/v) mixture of Hexane (Merck, Analar; Darmstadt, F.R.G.) and Diethyl ether (Merck, Analar; Darmstadt, F.R.G.). Dried extracts were dissolved in 0,1 ml phosphate buffer (pH = 7,0) containing 0,1% gelatine (Saarchem (Pty) Ltd, Muldersdrift, South Afica) and 0,1% sodium azide (Saarchem (Pty) Ltd)-(pH = 7,0). Standards ranging from 7,8 to 1000 pg androstenedione (Δ^4-Androstene-3,17-dione; Sigma Chemical Co., Dorset, U.K.) per 0,1 ml phosphate buffer and buffer blanks were included in duplicate in each assay. Antiserum raised against androstenedione-7-hemisuccinate-bovine serum albumin (Miles Yeda, Kiryat Weizman, Rehovot, Israel) in rabbits, and diluted to 13 ml in phosphate buffer was added (0,1 ml) to standards, reagent blanks and plasma extracts. To this was added TRK 454 ([1,2,6,7-^{3}H]Androst-4-ene-3,17-dione) tritiated androstenedione (Radiochemical Centre, Amersham, Bucks, U.K.) dissolved in assay buffer in 0,1 ml ($\sim$ 10 000 c.p.m.) aliquots. The contents of each tube were mixed and incubated overnight at 4°C. Separation of antibody-bound and free androstenedione was carried out at 4°C by adding 0,75 ml dextran-coated charcoal consisting of a suspension of charcoal (Aktivole; Merck, Darmstadt, F.R.G.) in assay buffer (0,156 g/ml) containing 0,0156 g Dextran T-40 (Phamacia, Uppsala, Sweden) to each tube.

These solutions were mixed gently for 30 sec, incubated at 4°C for 12 min and centrifuged at the same temperature at 1500 g for 10 min. The

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supernatants were decanted into scintillation vials and scintillation fluid (4,0 ml; Scintillator 299^{TM}; Packard Instrument Co.; Illinois, USA) was added to each vial. The contents of the vials were mixed well and radioactivity was measured at least 4 h later for 2 min, using a Packard 1500 Tri-Carb scintillation counter (Packard Instrument Co.; Illinois, USA).

Mathematical interpolation of recorded sample values against a standard curve was carried out using Securia^{TM} Plus RIA/QC software (Packard Instrument Co., Downers Grove, UK) over the range 7,8 - 1000 pg/tube. Recovery of known amounts of [1,2,6,7 - ^{3}H]Androst-4-ene-3,17 dione ($\sim$ 10 000 c.p.m.) in phosphate buffer to which pooled plasma were added, served to determine procedural losses incurred during extraction. Extraction efficiency and the original volume of plasma (0,1 ml) extracted were taken into account when calculating the concentrations of androstenedione in plasma samples.

Androstenedione status: As was the case for testosterone status estimation a number of parameter estimates were tested, namely: initial sample, immobilisation response and the LH-RH response. Procedures used were identical to those previously described for testosterone (see pg. 18).

Results

Androstenedione assay validation: Antiserum specificity was determined by the suppliers (Bio-Yeda). Cross-reaction with other steroids were as follows: 5 $\propto$ -Androstane-3,17-dione 32%, Testosterone and Dehydroepiandrosterone 3%, 11-Deoxycorticosterone and Progesterone 0,6%,

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Oestrone 0,2% and Oestradiol-17β < 0,01%. Sensitivity of the assays ranged from 3,92 to 16,70 pg/ml ($\bar{x}$ = 5,98 ± 3,63 s.d.; n = 7). Extraction efficiency ranged from 87,66 to 96,63% ($\bar{x}$ = 90,31; n = 7) and was unaffected by plasma volume (50 - 500 μl). Buffer blanks contained 14,81 ± 29,22 s.d. pg androstenedione equiv./ml (n = 7). The addition of 250, 500, 1000 pg androstenedione/tube to a plasma pool, resulted in recoveries of 99 ± 13% s.d. over the range. Parallelism was evident over the entire range of the standard curve, and intra- and inter-assay coefficients of variation were 7,31% and 5,28% respectively.

Androstenedione status: The androstenedione profiles for all animals subjected to serial sampling are shown in Fig. 6. As was the case for testosterone few of the profiles conformed to classical theoretical patterns, suggesting that more than one organ contributed to the observed response (see Lindeque et al. 1986). Similar to the result obtained in the testosterone profiles (Fig. 2) there is a positive relationship between the different parameter estimates of androstenedione status (Table 2). The significant correlation between these three parameter estimates, namely, the initial sample, the immobilisation response and the LH-RH response indicates that either of these indices may be a relatively accurate estimate of the androstenedione status of spotted hyaenas. The initial sample was therefore employed to enlarge the effective sample size and to allow direct comparison between different age groups.

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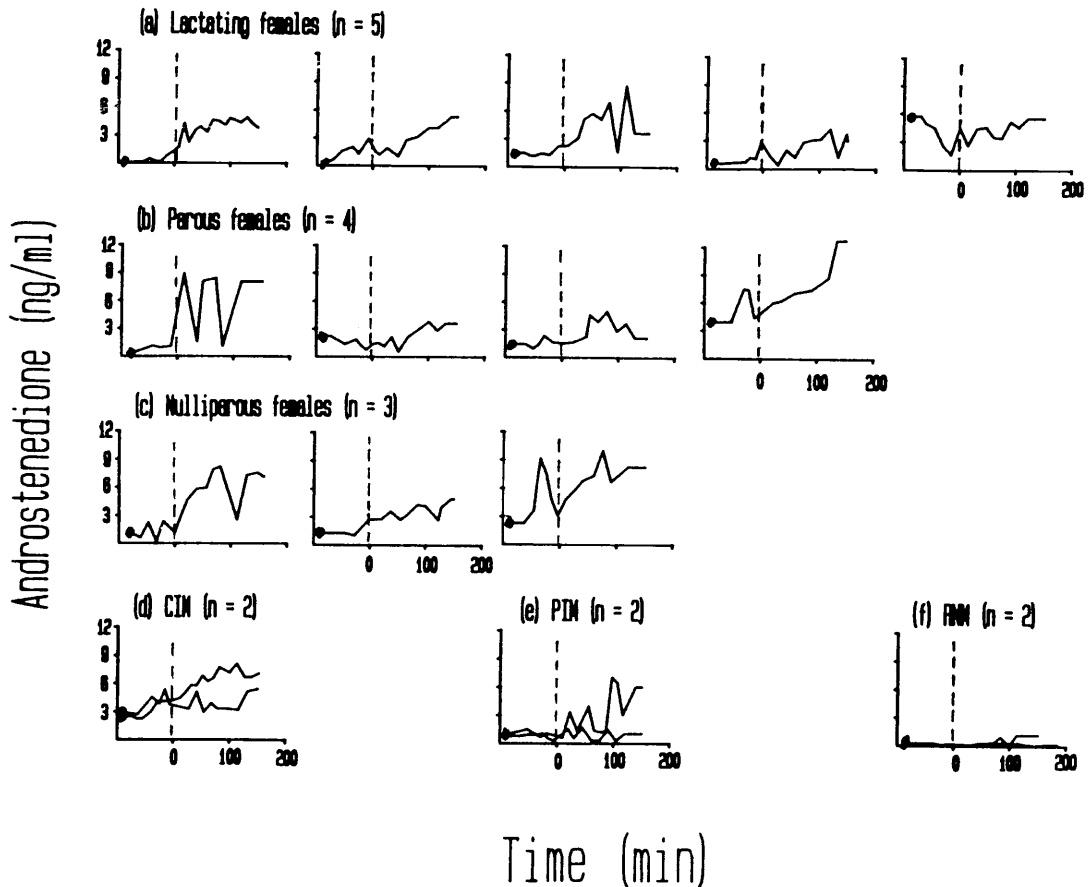
FIG. 6. Androstenedione profiles for 18 spotted hyaenas anaesthetised with Halothane following Zoletil immobilisation. The three androstenedione status parameter estimates, namely, initial sample (●), immobilisation response (-90 to 0 min) and LH-RH response (0 to 120 min are indicated. (a) Lactating females, (b) parous females, (c) nulliparous females, (d) central immigrant males, (e) peripheral immigrant males and (f) resident natal males.

Although no significant rank correlation between age and the initial androstenedione sample could be demonstrated for all animals older than one year (r = 0,31; P > 0,05; n = 32), it appears that any positive correlation was mainly due to the male component of the sample (r = 0,57; P < 0,05; n = 15) as initial female androstenedione samples showed no relationship to age (r = 0,01; P > 0,1; n = 17). Again, as was the case with testosterone it seems that the initial androstenedione sample may reflect some

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TABLE 2. Calculated relationships between the three androstenedione status parameter estimates in the spotted hyaena (n = 18). All estimates were collected from the same animals during a single immobilisation. SAS PROC CORR SPEARMAN (r_S).

Parameter estimates	Spearman's coefficient (r_S)	Significance level
1. Initial sample *vs* immobilisation response	0,91	P < 0,001
2. Initial sample *vs* LH-RH response	0,55	P < 0,05
3. Immobilisation response *vs* LH-RH response	0,66	P < 0,05

developmental process in male spotted hyaenas over one year of age. This is supported by the changing ratio of testosterone : androstenedione during the developmental process. Without exception immature males had a testosterone : androstenedione ratio less than one ($\bar{x}$ = 0,25 $\pm$ 0,22; n = 17), whereas 66% of the mature males, including all the CIMs, had a ratio greater than one ($\bar{x}$ = 1,79 $\pm$ 1,74; n = 15). In females androstenedione was the dominant hormone with only two adult females (7%) showing a testosterone : androstenedione ratio greater than one. In addition, it was only in the male data subset that a significant positive correlation between plasma testosterone and androstenedione concentrations was recorded (r = 0,75; P < 0,01; n = 15). Furthermore, as androstenedione is generally a precursor for testosterone synthesis (Baxter & Tyrell 1987), the lack of any significant difference in circulating androstenedione between CIMs and female cubs (Fig. 7b) is not surprising.
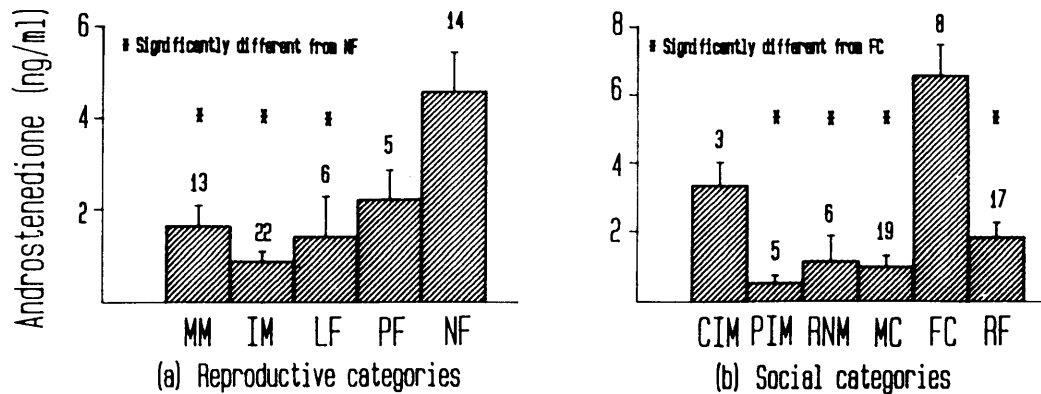
FIG. 7. Mean ($\pm$ s.e.m.) peripheral androstenedione titres for:

(a) <u>Reproductive categories</u>: MM = mature males (> 2 yrs), IM = immature males ($\leq$ 2 yrs), PF = parous females, LF = lactating females, NF = nulliparous females, and

(b) <u>Social categories</u>: CIM = central immigrant males, PIM = peripheral immigrant males, RNM = resident natal males, MC = male cubs, FC = female cubs and RF = resident females. ANOVA was run on (androstenedione)^{0,5}. Statistical significance was measured at the level of P < 0,05.

Non parametric analysis of variance (PROC NPAR1WAY - Kruskal-Wallis; Chi-square approx.; X_1 = 10,62; P < 0,05) revealed that females had higher mean peripheral androstenedione levels than males (3,30 $\pm$ 0,59 cf 1,14 $\pm$ 0,22 s.e.m. ng/ml; n = 60), when calculated from initial samples. Parametric ANOVA (PROC GLM) comparing (androstenedione)^{0,05} titres for the different reproductive categories of spotted hyaenas (Fig. 7a) showed significant differences between groups ($F_{0,05[4,55]}$ = 7,39). A comparison of means (Tukey's studentised range - HSD) revealed that nulliparous females had

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significantly higher (HSD_{55} = 3,99; P < 0,05) mean androstenedione levels than all other reproductive categories, with the exception of the parous females (Fig. 7a). Similar analysis of variance of (androstenedione)^{0,05} among the different social categories also revealed significant variance ($F_{0,05[5,52]}$ = 72,39) between categories. Here female cubs ($\leq$ 1 yr) had significantly higher (HSD_{52} = 4,184; P < 0,05) androstenedione concentrations than all other social categories except the central immigrant males (Fig. 7b).
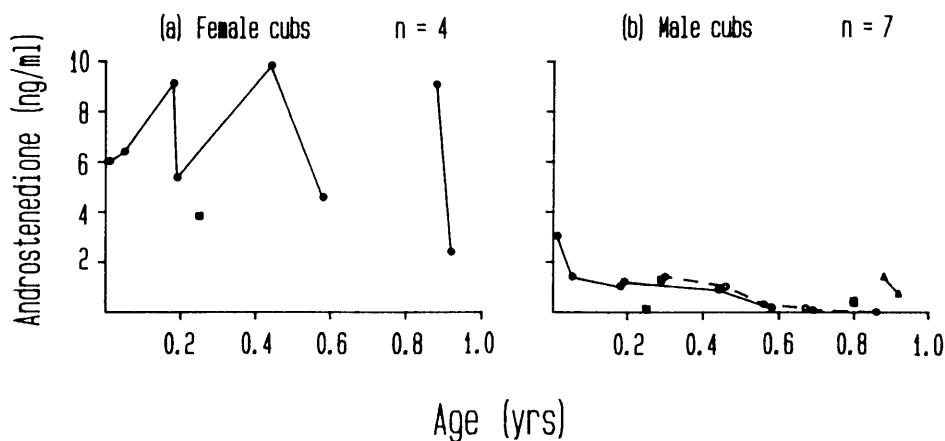
FIG. 8. Plasma androstenedione concentration profiles for (a) female and (b) male cubs. Serial samples taken from the same animals are connected.

A comparison of means between male and female cubs illustrated that female cubs had significantly higher androstenedione concentrations than male cubs (Fig. 7). This trend is also clear from the developmental profiles for cub plasma androstenedione concentrations (Fig. 8). Observed plasma androstenedione concentrations in cubs showed no correlation with age (r = -0,31; P > 0,1; n = 28). However, the male data subset showed a significant

negative correlation (r = -0,53; P < 0,05; n = 19) with age, indicating decreased androstenedione synthesis during the neonatal developmental stages.

Discussion

Initial investigations into an endocrine basis for female dominance in the spotted hyaena found it difficult to separate the sexes purely on the basis of peripheral testosterone concentrations (Racey & Skinner 1979, Lindeque *et al.* 1986). This was presumed to represent high female androgens, which would explain the dominance of females as a class over males (Racey & Skinner 1979). On the other hand, comparative analyses of the peripheral testosterone titres for all the hyaena species suggested that the inability to distinguish between male and female spotted hyaenas may be due to low male testosterone levels, rather than high female titres (Van Jaarsveld & Skinner 1987). This raised the possibility that testosterone concentrations in males of this species are largely determined by factors other than the sex of the individual (see Keverne 1987).

The analysis of sex-specific peripheral testosterone concentrations obtained in this investigation confirm earlier reports that it is difficult to distinguish between the sexes (Racey & Skinner 1979, Lindeque *et al.* 1986). However, a comparison between different reproductive categories revealed that mature males had higher testosterone titres than all reproductive categories, except parous females. This, together with the observation that parous females had higher testosterone concentrations than immature males (Fig. 3a), suggest that this species has a larger variance in circulating testosterone titres within the sexes than between them.

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Furthermore, a comparison between different social classes (Fig. 3b) illustrates that most of the male variance can be attributed to the high testosterone titres of the mating or central immigrant males (CIM), while all the other male social categories have testosterone levels indistinguishable from those found in females. This observation explains earlier macroscopic and histological reports that a significant proportion of males in the potentially reproductively active age groups are apparently incapable of breeding (Matthews 1939, Lindeque *et al.* 1986).

Although there is a tendency for plasma testosterone to increase with age in males, the testosterone profiles for the various social categories (Fig. 3b) provides strong evidence in favour of social suppression of male peripheral testosterone concentrations in this species. Only those males that have been accepted into target clans after dispersal (CIM - Henschel & Skinner 1987) have testosterone levels on a par with those recorded in both male striped hyaenas (*Hyaena hyaena*) and brown hyaenas (*H. brunnea*) - (see Van Jaarsveld & Skinner 1987). At the same time the social category which represents the period of increased social stress, namely the PIMs, that are dispersing from their natal clans to target clans (Henschel 1986), appear to have depressed testosterone concentrations (Fig. 3b) . This pattern of changing plasma testosterone titres in male spotted hyaenas does therefore not merely reflect maturative aspects of development but is clearly also dependent on the experiential development of an individual. In contrast, plasma testosterone concentrations in females do not reflect either maturative or experiential aspects of development (Fig. 3b), and seem to be largely determined by changes in ovarian activity (Fig. 3a).

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These findings partly support previous data from an East African population where males had higher testosterone levels than females (Glickman *et al.* 1987) and females had total androgen levels significantly lower than males (Frank *et al.* 1985a). Although the findings by Frank *et al.* (1985a) that androgen levels were highest in the alpha -individuals of both sexes is not contradicted by this investigation, care should be exercised on two points: first, Henschel & Skinner (1987) illustrated that the CIMs are not necessarily the same as the alpha - males, as the latter may be RNMs due to their inherited dependent rank; and secondly, it now appears that the stage of ovarian activity at the time of sampling will affect the testosterone concentrations obtained in females.

The data presented in this study illustrate that any analysis of the sex-specific distribution of testosterone for <u>Crocuta</u> will be affected by the dispersal and reproductive stages, as well as the ages of the animals included in the sample. This probably accounts for the contradictory results reported on this subject to date (Racey & Skinner 1979, Frank *et al.* 1985a, Lindeque *et al.* 1986). No significant difference was observed in mean peripheral testosterone concentrations (Fig. 3b) among male and female cubs. On the other hand, a profile of age specific plots (Fig. 4) suggests that further investigation at the neonatal stages may prove informative, as a single sample obtained from a four day old male approached mean adult male and female concentrations. A notable feature of the genitals of this young animal was that testes were palpable in the scrotum; a feature which had disappeared by 18 days and later reappeared at an age of five months. This increased neonatal testosterone secretion associated with a temporary descent of the testis conforms to established patterns of testosterone

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secretion in other mammals (Santen 1987) and provides an easy method of sexing cubs at these very young ages.

One further issue which confounds the interpretation of the biological availability of peripheral testosterone in spotted hyaenas, is the high variability in plasma testosterone binding observed in some adult females (Fig. 5). Although it is suspected that both this increase and variability in plasma testosterone binding is due to the influence of ovarian activity on a plasma binding globulin (Burke & Anderson 1972, Chapter 4), this issue will require further investigation. Nevertheless, these preliminary results indicate that the biological availability of testosterone to adult females may be significantly lower than would be deduced from an analysis of organically extracted plasma samples.

Androstenedione was initially included in reports on the androgen status of spotted hyaenas as data in support of the previously mentioned findings related to peripheral testosterone concentrations between the sexes. However, although it is now clearly established that both the adrenals and gonads of male and female spotted hyaenas are involved in the secretion of testosterone as well as androstenedione (Lindeque *et al.* 1986), the specific roles played by these two hormones during development is poorly defined. Moreover, studies have shown a distinct temporal discrepancy in the peripheral availability of both testosterone and androstenedione during the development of spotted hyaenas. Data presented here show that it is only in males that any positive correlation between plasma testosterone and androstenedione exists. This suggests that the availability of androstenedione, which is a precursor hormone for testosterone, oestradiol-17ß and oestrone (Baxter & Tyrrell 1987), in adult females may be related

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to oestrogenic rather than androgenic activity. This view is supported by the positive correlation observed between plasma androstenedione and oestradiol-17β in adult females (see Chapter 4).

Although androstenedione is generally acknowledged to have weaker androgenic effects than testosterone (Baxter & Tyrrell 1987), it has emerged that periods of abundance of this hormone in the peripheral circulation may play a vital role during the socio-ecological development of spotted hyaenas. Studies to date (Racey & Skinner 1979, Lindeque *et al.* 1986, Glickman *et al.* 1987), together with the data presented here indicate that peripheral androstenedione titres are highest in nulliparous females (< 3 yrs) - (Fig. 7a), a trend that is accentuated when female cubs are compared with all other social categories (Fig. 7b). This observation, together with the lack of any recorded sex-related difference in peripheral androstenedione concentrations during early fetal development (Lindeque & Skinner 1982a) or adult stages (Racey & Skinner 1979, Lindeque *et al.* 1986, Glickman *et al.* 1987, this study) suggest that the biggest discrepancy in androstenedione titres occurs in the late stages of gestation or the neonatal period (Glickman *et al.* 1987). Data presented here illustrate that this period of elevated androstenedione concentrations in female cubs and nulliparous females extends at least from four days after birth to three years of age (see Fig. 8), and are in agreement with earlier reports that elevated androstenedione concentrations may provide the key to the behavioural dominance of female over male spotted hyaenas (Glickman *et al.* 1987).

Contradictory results seem to have emerged regarding the possible origins of androstenedione in sub-adult females: Racey & Skinner (1979) found high

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levels of androstenedione in the adrenal glands of a nulliparous female while ovariectomy resulted in a marked decline in plasma androstenedione of infant and adult hyaenas (Glickman *et al.* 1987) as well as total androgens (Frank *et al.* 1985b). However, non-invasive pituitary challenge techniques showed marked increases of peripheral androstenedione in response to both exogenous LH-RH and ACTH stimulation in a sub-adult female but not in a parous female (Lindeque *et al.* 1986). These seemingly contradictory results support the hypothesis of an adrenal and ovarian contribution to peripheral androstenedione in females, rather than purely an ovarian origin as proposed by Glickman *et al.* (1987).

The increase in the ratio of testosterone : androstenedione to values greater than one for the majority of mature males supports an earlier report by Lindeque (1981), and conforms to the general mammalian pattern originally described for *Bos taurus* (Skinner, Mann & Rowson 1968). The interesting aspect that emerged in the present study is that all the CIMs had a testosterone : androstenedione ratio greater than one.

CHAPTER 4 - OVARIAN ACTIVITY

Although endocrine correlates of the spotted hyaena ovarian cycle are unavailable, a number of attempts have been made to distinguish between different reproductive stages in this species using plasma steroid concentrations (Racey & Skinner 1979, Lindeque 1981, Gombe 1985). Cyclic ovarian events represent a considerable component of female endocrine dynamics, more so in a polyoestrous species such as the spotted hyaena. It is therefore essential that the effects of such ovarian activity, be they cyclic, anoestrus or in the luteal stage, on the total hormonal *milieu* be assessed. The cycle phase not only alters gonadotrophin levels and thereby steroid levels; but also modulates other hormone levels and their responses. The ultimate biological activity is thus determined by the relative proportions of all circulating hormones, together with the specific receptivity of the target organs (Baird 1972).

Hormonal interrelationships also influence their physiological effect. Some hormones have synergistic actions (e.g. catecholamines and thyroid hormones) whereas others have antagonistic peripheral actions (e.g. testosterone and oestradiol-17ß). Therefore the net physiological effect is dependent on the levels of the agonist and antagonist, and not simply on the concentrations of the circulating hormones (Ismail 1981). For the above reasons any enquiry into the existance of possible endocrine correlates of social development would not be representative were the emergence of ovarian activity not included in the final analysis. The effect of this emergent ovarian activity on the total female hormonal *milieu* was monitored by measuring the concentrations of the steroids progesterone and oestradiol-17ß respectively.

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Progesterone

Introduction

Following Matthews' (1939) demonstration that lactating females could easily be identified through external examination in the field, Racey & Skinner (1979) demonstrated that pregnant hyaenas could be positively identified because of their high progesterone titres (~56,92 ng/ml) when compared with lactating females (~4,06 ng/ml). These findings were subsequently confirmed by Lindeque (1981). Gombe (1985), on the other hand, could not distinguish between a pregnant and a non-pregnant female using similar radio-immunoassay techniques. Plasma progesterone determinations were carried out in the present study to evaluate the variation between the different reproductive categories of female spotted hyaenas.

Methods

Progesterone assay: Progesterone was assayed in plasma extracts according to the procedures described by Van Aarde (1985) with the following modifications: 0,1 ml plasma samples were diluted to achieve between 20 and 40% specific binding using phosphate buffer (pH 7,0) before duplicate 0,1 ml aliquants of the diluted plasma were extracted by the addition of 4,0 ml petroleum ether (Saarchem (Pty) Ltd, Krugersdorp, South Africa) and mixing the contents for five one min periods with one min intervals on a vortex mixer. Procedural losses incurred during extraction were estimated by the recovery of known amounts of (~ 10 000 c.p.m.)[1,2,6,7-^{3}H]progesterone (Radiochemical Centre, Amersham, Bucks, U.K.) in phosphate buffer. Antisera raised in rabbits against progesterone-6-bovine serum albumin and provided

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by R P Millar (University of Cape Town) were added to plasma extracts, buffer blanks, ether blanks and standards at a dilution of 1:4000. Progesterone bound to the antibody was separated from free steroids by the addition of 0,75 ml dextran coated charcoal (0,78% activated charcoal, Sigma, Dorset, U.K.; 0,078% dextran T40, Pharmacia, Uppsala, Sweden) at 4^oC.

Results

Progesterone assay validation: The specificity of the antisera was quantified by the supplier (R P Millar, Department of Chemical Pathology, University of Cape Town, South Africa) and cross-reactivity with other steroids were as follows: 17α-hydroxyprogesterone, 2,6%; 11α-hydroxyprogesterone, 48,3%; 11α-hydroxyprogesterone, 6%; 5α-pregnane-3-20-dione, 25,1%; pregnenolone, 5,2%; 3α-hydroxy-5-pregnane-20-one, 0,4%; 20α-hydroxy-4-pregnane-3-one, 0,3%; 11-deoxycorticosterone, 1,9%; 11-deoxycortisol, 1,7%. Cross reaction of cortisol was less than 0,1%, and that of testosterone, Δ^4-androstenedione, oestradiol-17ß and oestrone less than 0,001%. Sensitivity of the assays ranged from 18,0 to 34,0 pg/ml ($\bar{x}$ = 0,026 $\pm$ 0,008 s.d.; n = 3). Three buffer blanks measured during the assays contained 0,14 $\pm$ 0,19 s.d. ng progesterone equiv./ml.

Recovery estimates ranged from 72,8 to 85,5% ($\bar{x}$ = 80,3%; n = 3). Results were corrected accordingly. Intra- and interassay coefficients of variation were 8,96% and 8,56% respectively. Recovery estimates were unaffected by plasma volume. The addition of 31,2, 62,5 and 125,0 pg of progesterone to male plasma resulted in recoveries that did not differ significantly from expected values (t_2 = 3,97; P > 0,05). Parallelism could only be

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demonstrated between 20 and 40% binding, and samples that fell below this effective sensitivity were designated values of zero. All other samples were diluted with phosphate buffer to obtain the required binding and corrected accordingly.
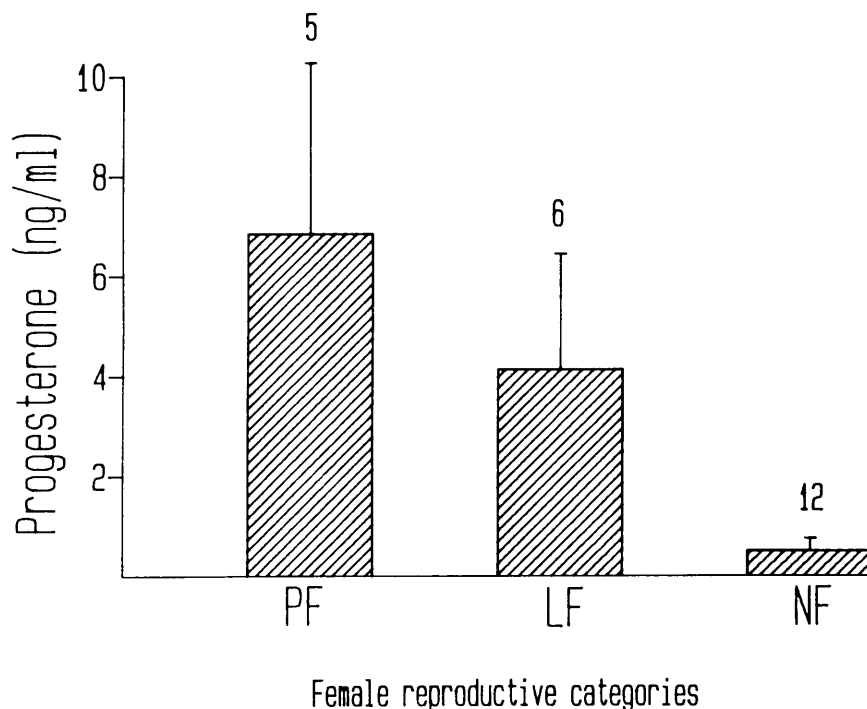
Fig. 9 Mean ($\pm$ s.e.m.) for the three reproductive categories in which measurable progesterone concentrations were recorded. PF = parous female, LF = lactating female and NF = nulliparous female. * indicates a significant difference at P < 0,05.

Progesterone status: Measurable progesterone titres were only observed in the following reproductive categories: parous females, lactating females and nulliparous females. The lack of any recorded progesterone levels in males and cubs of both sexes could be the result of low biological concentrations, or the inability of the antiserum to effectively measure low progesterone concentrations (see assay validation). The observed

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progesterone levels for the three female reproductive classes, depicted in Fig. 9 shows that ANOVA (PROC GLM; $F_{0,05[2,11]}$ = 1,64) revealed no significant variation in circulating progesterone titres between groups. Progesterone concentrations in adult females were positively correlated to plasma testosterone (r = 0,52; P < 0,05; n = 17) and plasma androstenedione concentrations (r = 0,53; P < 0,05; n = 17).

Discussion

The observed correlation between plasma progesterone, androstenedione and testosterone concentrations is probably indicative of increased steroid synthesis in general, as progesterone serves as a precursor for both androstenedione and testosterone (Baxter & Tyrrell 1987). These above data, although based on a limited sample size, indicate that the variance of circulating progesterone within groups is larger than the variance between groups, implying that the females may not be assigned to any of the three reproductive categories purely on the basis of circulating progesterone titres. This result is in accordance with an earlier study to this effect (Lindeque 1981), in which a combination of progesterone, oestradiol-17ß and oestrone was required to effectively distinguish between females of different reproductive states. Furthermore, the inability to distinguish between a pregnant and a non-pregant spotted hyaena using peripheral progesterone concentrations (Gombe 1985) gives further support to the emerging pattern of large variations in circulating progesterone values within the respective reproductive states. The implications of this are that although females in different states of ovarian activity, as determined through morphological and/or histological methods may be found to be hormonally distinct from other reproductive categories, the reverse

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process in which the ovarian activity of an individual may be inferred from its hormonal *milieu* is not yet possible. This emphasises our poor understanding of the hormonal changes associated with cyclic ovarian activity in this species.

A further complicating factor seems to be our inability to distinguish lactating females from non-lactating females using circulating progesterone concentrations (Fig. 9). Although this is further support for earlier reports that lactating individuals may be pregnant at the same time (Lindeque 1981), the absence of a distinct lactational anoestrus in a number of individuals investigated to date (Lindeque 1981, present study) makes the desription of steroid correlates for the cyclic ovarian pattern even more problematic.

Oestradiol-17ß

Introduction

The pattern of oestradiol-17ß secretion in this species is less clear than that found in progesterone. The only marked feature of published results is the relatively high oestradiol-17ß titres found in pregnant females compared to the other groups (Racey & Skinner 1979, Lindeque 1981). However, Gombe (1985) was unable to differentiate between a pregnant and a non-pregnant individual using oestradiol-17ß titres. The distribution of peripheral plasma oestradiol-17ß concentrations for the respective social and reproductive categories were analysed, and then related to observed correlations between plasma hormone concentrations in order to gain some clarity concerning the nature of the ovarian dynamics of this species.

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Methods

Oestradiol-17ß assay: Oestradiol-17ß was assayed following Van Aarde (1985), and using the following modifications: duplicate plasma samples (0,5 ml) were extracted in 4 ml diethyl ether (Merck, Darmstadt, FRG) by shaking on a multi-tube vortexer five times for one min. Antisera were raised in rabbits against Oestradiol-6-(O-carboxymethyl) oxime-bovine serum albumin conjugate.

Results

Oestradiol-17ß assay validation: Antisera specificity was quantified by the supplier (R P Millar, Department of Chemical Pathology, University of Cape Town, South Africa) and cross reactions with other steroids were as follows: oestrone 0,01%; pregnanediol, corticosterone, deoxycorticosterone, 17α-hydroxypregnenolone, androstenedione, 20α-dihydroprogesterone, progesterone, testosterone and cortisol < 0,001%. Sensitivity of the assays ranged from 10,86 - 19,50 pg/ml ($\bar{x}$ = 16,04 $\pm$ 3,62 s.d.; n = 5). Extraction efficiency ranged from 86,50 - 92,47% ($\bar{x}$ = 88,71; n = 5) and was unaffected by plasma volume (200 - 1000 μl). Results were corrected accordingly. Buffer blanks contained 9,24 $\pm$ 3,1 s.d. pg oestradiol-17ß equiv./ml (n = 5). The addition of oestradiol-17ß (62,5, 125 , 250 pg) to a plasma pool resulted in recoveries of 115%, and parallelism was demonstrated over the entire range of the standard curve. Intra- and inter-assay coefficients of variation were 12,5% and 2,5% respectively.

Oestradiol-17ß status: Analysis of rank correlation revealed a significant increase with age for the sample as a whole (r = 0,48; P < 0,01; n = 31). This can be attributed to the high rank correlation between age and plasma

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oestradiol-17ß titres in females ($r = 0,59$; $P < 0,05$; $n = 17$). Males showed no age related correlation with oestradiol-17ß. Sex-specific analysis of oestradiol-17ß values showed a significant difference (PROC NPARANOVA; Kruskal-Wallis Chi-square approx.; $X_1 = 0,012$; $P < 0,05$) between male ($\bar{x} = 15,30 \pm 2,86$ s.e.m.; $n = 29$) and female ($\bar{x} = 34,87 \pm 8,23$ s.e.m.; $n = 24$) values.

Comparisons of measured oestradiol-17ß concentrations between the various reproductive and social categories are shown in Fig. 10. Non parametric analyses of variance revealed significant differences within both reproductive (PROC NPARANOVA; Kruskal-Wallis Chi-square approx.; $X_4 = 0,029$; $P < 0,05$) and social (PROC NPARANOVA; Kruskal-Wallis Chi-square approx.; $X_5 = 0,043$; $P < 0,05$) categories. Comparison of means could however not be carried out due to non normally distributed data sets. Visual inspection of Fig. 10 (a + b) shows that there is a tendency for parous, lactating and resident females to have higher oestradiol-17ß levels than the other reproductive and social categories respectively. Moreover, parous females generally seem to have higher plasma oestradiol-17ß concentrations than lactating females.

The relationship between plasma oestradiol-17ß concentrations and the levels of the other gonadal steroids followed predictable trends. In adult females oestradiol-17ß was positively correlated with two of its biosynthestic precursors, namely progesterone ($r = 0,74$; $P < 0,001$; $n = 17$) and testosterone ($r = 0,54$; $P < 0,05$; $n = 17$). Similarly, in adult males
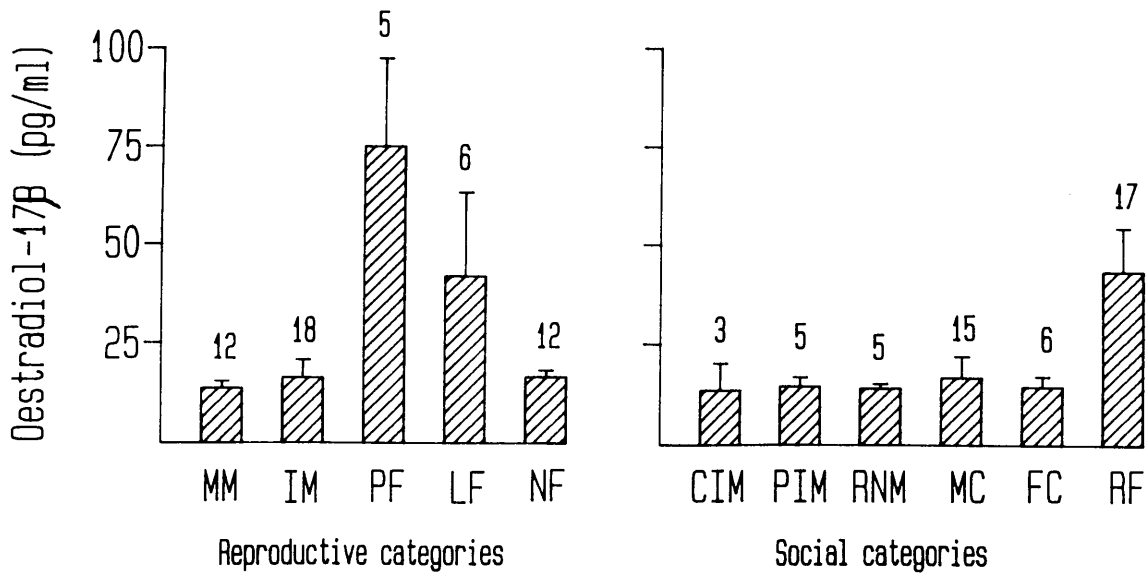
Fig. 10. Mean (± s.e.m.) oestradiol-17ß concentrations for (a) reproductive categories, and (b) social categories respectively. MM = mature males, IM = immature males, PF = parous females, LF = lactating females, NF = nulliparous females, CIM = central immigrant males, PIM = peripheral immigrant males, RNM = resident natal males, MC = male cubs, FC = female cubs and RF = resident females.

plasma oestradiol-17ß titres were also closely related with those of its biosynthestic precursors: it had a positive correlation with plasma androstenedione (r = 0,77; P < 0,01; n = 14) and nearly so with testosterone (r = 0,51; P = 0,06; n = 14). In contrast plasma oestradiol-17ß titres showed no relationship with other gonadal steroids in cubs, however a negative correlation was recorded with age for male cubs (r = - 0,70; P < 0,01; n = 15). This is probably a result of the increased steroid synthesis at neonatal stages described earlier for testosterone. In adult females there was also a positive correlation between plasma oestradiol-17ß and age (r = 0,60; P < 0,05; n = 17).

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<u>Oestradiol-17β and plasma testosterone binding</u>: Plasma testosterone binding showed a positive correlation with both plasma progesterone (r = 0,73; P < 0,001; n = 25) and oestradiol-17β (r = 0,65; P < 0,001; n = 25) concentrations for the sample as a whole. Closer investigation revealed that these patterns were due solely to the strong positive correlation that exists between plasma testosterone binding and circulating steroids such as oestradiol-17β (r = 0,81; P < 0,001; n = 14); progesterone (r = 0,67; P < 0,01; n =14) and testosterone (r = 0,57; P < 0,05; n = 15) in adult females. Males on the other hand showed no correlation between plasma testosterone binding and circulating steroid concentrations.

Discussion

Increased oestradiol-17β concentrations with age are indicative of increased steroid synthesis associated with the commencement of ovarian activity in females. This is supported by the low titres recorded in nulliparous females when compared with the higher oestradiol-17β levels recorded in the parous and lactating reproductive categories (Fig. 10a), as well as the positive correlation with age. In addition, the lower oestradiol-17β concentrations observed in lactating than parous females are indicative of oestradiol-17β suppression during lactation in this species. However, the presence of significant plasma oestradiol-17β titres in lactating females suggests that some ovarian activity may occur during this reproductive stage, and supports earlier observations of females that are both lactating and pregnant (Lindeque 1981, present study). This supports the conclusion reached earlier in the progesterone analysis that there is no clearly defined lactational anoestrus in the spotted hyaena. Moreover, the large variances recorded within the parous and lactating reproductive

categories indicate that these reproductive categories, originally based on morphological criteria, are of insufficient resolution to elucidate the nature of spotted hyaena ovarian dynamics.

Lindeque (1981) found significant differences in circulating steroids that enabled a differentiation between the following reproductive states: nulliparous females exhibiting total ovarian inactivity, and those with developing follicles; parous lactating females with developing follicles and those without; parous pregnant and parous anoestrous females. These reproductive classes were defined in retrospect after histological confirmation and no concrete predictive criteria for an alternative classification, based purely on peripheral steroid concentrations emerged from that investigation. For this reason, the easily discernable reproductive classes, originally defined by Matthews (1939), and confirmed by Lindeque (1981), were retained for comparative purposes in this study.

The strong positive correlation observed between plasma testosterone binding ability and circulating steroid concentrations in adult females supports earlier observations that plasma binding may be related to ovarian activity (pg. 34). Also, increased plasma testosterone binding coupled with increases in circulating steroids could decrease the biological availability of plasma steroids, including testosterone to adult females (Burke & Anderson 1972, Baxter, Frohman, Broadus & Felig 1987). This would further reduce the androgenic effects of circulating androgens in adult females and offers additional support for the hypothesis that female dominance in this species is not the result of high concentrations of circulating androgens in adult females (Van Jaarsveld & Skinner 1987). The observed significant correlations between plasma testosterone binding and

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the recorded progesterone and testosterone concentrations, are probably the result of the high correlations that these biosynthestic precursors (Baxter & Tyrrell 1987) had with plasma oestradiol-17ß titres.

CHAPTER 5 - ADRENO-CORTICAL STATUS

Introduction

The adrenal cortex of animals seems to be activated by a wide range of inputs which may collectively be categorised as stressors (Mason 1968, Levine 1971, Brain 1979, Baxter & Tyrrell 1987). The adrenal cortex is stimulated to produce and release cortisol as well as other adrenal steroids, including androgens and aldosterone, under the influence of the hypothalamus and the pituitary gland. This neuro-endocrine axis acts *via* the action of corticotrophin releasing factor (CRF), arginine vasopressin and possibly other substances, released by the hypothalamus, stimulating adrenocorticotropin (ACTH) secretion from the anterior pituitary gland. ACTH in turn stimulates the release of cortisol and other steroids by the adrenal cortex.

Three mechanisms operate to control cortisol release by the adrenal cortex. First, endogenous rhythms in the brain cause a pulsatile ACTH secretion which results in episodic, circadian and meal stimulated patterns. Second, a number of environmental inputs or stressors, both physical and emotional, can elevate ACTH and cortisol secretion above the mentioned pulsatile pattern. Lastly, ACTH release is suppressed by a feedback action of plasma cortisol on both the hypothalamus and pituitary gland. (Baxter & Tyrrell 1987). Besides the short-term influences of ACTH on adrenal steroid production it also has a trophic influence on the adrenal which determines the magnitude of steroid hormone responsiveness to an acute elevation of ACTH. Short-term influences are restricted to an increase of cortisol from the cortex within two to three minutes, due largely to increased cortisol

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synthesis (Gill 1979, Simpson & Waterman 1983, Hall 1985). With prolonged stimulation, hypertrophy which includes increased adrenal cortex weight, and increases in protein and nucleic acid content, can result in the doubling of gland size (Baxter & Tyrrell 1987). It is this long-term response of the adrenal cortex to continuous ACTH stimulation which reportedly plays a significant role in shaping the socio-ecological development of an animal. However, acute psychological stressors show a large variance in the degree of cortisol response (Baxter & Tyrrell 1987) making it difficult to predict the effect of social stress on adrenal cortex responsiveness. The above rationale for the functioning of the adrenal-neuro-endrocrine axis does suggest that animals exposed to environmental stressors should show a variable response to an induced stressor. However, numerous experiments that attempted to measure individual differences in the glucocorticoid stress response have revealed seemingly contradictory results.

Manoque, Candland & Leshner (1975) and Sapolsky (1983a) found lower basal cortisol levels in high-ranking squirrel monkeys (*Saimari sciuraus*) and olive baboons, while Golub, Sassenrath & Goo (1979) showed that low basal cortisol levels in weanling rhesus monkeys (*Macaca mulatta*) could be predictive of dyadic (paired) dominance. In addition, Manoque *et al.* (1975) and Sapolsky (1983a) showed that high-ranking subjects had a greater relative rise in response to stress. Another body of literature, however, supports an opposite view namely that dominant primates have heavier adrenals (Hayama 1966) and display smaller increments in cortisol levels following stress (Coe, Mendoza & Levine 1979). The more consistent picture that has emerged from the rodent literature is in agreement with the first view (Davis & Christiaan 1957, Barnett 1963, Levine & Mullins 1966, Louch &

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Higginbotham 1967). Most of these studies have however been restricted to captive animals or laboratory bred individuals and it is not yet conclusively established whether or how these mechanisms operate under natural conditions. One such study on a natural population (Sapolsky 1982, 1983a, 1985) indicated that stress-induced suppression of gonadal function occurs in the olive baboon, and implicated glucocorticoid action on the testes as a regulating mechanism. Moreover, dominant males with high copulation rates showed the lowest initial cortisol levels but showed larger and faster cortisol responses following stress (Sapolsky 1982, 1983a). This response was evidently the result of an accelerated secretion of ACTH by the pituitary gland (Sapolsky 1983b). In order to evaluate the role played by the adrenal axis during the socio-ecological development of the spotted hyaena, the pattern of cortisol responsiveness in the various reproductive and social categories were investigated.

Methods

Cortisol assay: Plasma cortisol was assayed by radio-immunoassay in triplicate following dilution of 0,1 ml plasma to 4 ml with phosphate buffer (pH = 7,0) containing 0,1% gelatine [Saarchem (Pty) Ltd, Muldersdrift, South Africa] and 0,1% sodium azide (Saarchem). Diluted aliquots (0,1 ml) were extracted by vortexing five times for one min together with 4 ml dichloromethane (Analar; BDH Chemicals Ltd, Poole, England). Tubes were left for 10 min to allow separation of organic and inorganic layers. After aspiration of the organic layer the extracts were dried under nitrogen. Dried extracts were reconstituted in 0,1 ml phosphate buffer. Standards ranging from 15,6 to 2000 pg cortisol (11,17∝,21-Trihydroxypregn-4-ene-3,20-dione; Sigma, Chemical Co., Dorset, U.K.) per 0,1 ml phosphate buffer,

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and buffer blanks were included in duplicate in each assay. Antiserum raised against cortisol-21-hemisuccinyl-thyroglobulin conjugate (BioMakor, Kiryat Weizman, Rehovot, Israel) in rabbits, and diluted to 20 ml in phosphate buffer was added (0,1 ml) to standards, reagent blanks and plasma extracts. To this was added [TRK 407; 1,2,6,7-^{3}H Cortisol] tritiated cortisol (Radiochemical Centre, Amersham, Bucks, U.K.) dissolved in phosphate buffer in 0,1 ml aliquots. The contents of each tube were mixed and incubated overnight at 4°C. Separation of antibody-bound and free cortisol was carried out at 4°C by adding 0,75 ml dextran coated charcoal consisting of a suspension of charcoal (Aktivole; Merck, Darmstadt, F.R.G.) in phosphate buffer (0,156 g/ml) containing 0,0156 g Dextran T-40 (Pharmacia, Uppsala, Sweden) to each tube.

These solutions were mixed gently for 30 sec, incubated at 4°C for 12 min and centrifuged at the same temperature at 1500 g for 10 min. The supernatants were decanted into scintillation vials and scintillation fluid (4,0 ml; Scintillator 299^{TM}; Packard Instrument Co., Illinois, USA) was added to each vial. The contents of the vials were thoroughly mixed and radioactivity was measured at least 4 h later for 2 min, using a Packard 1500 Tri-Carb scintillation counter (Packard Instrument Co., Illinois, USA).

Mathematical interpolation of recorded sample values against a standard curve was carried out using Securia^{TM} and RIA/QC software (Packard Instrument Co., Downers Grove, UK) over the range of 15,6 - 2000 pg/tube. Recovery of known amounts of tritiated cortisol (~ 10 000 c.p.m.) in phosphate buffer to which pooled plasma was added served to determine procedural losses incurred during extraction. Extraction efficiency, plasma

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dilution and the original volume of plasma (0,1 ml) extracted were taken into account when calculating the concentration of cortisol in plasma samples.

<u>Cortisol status</u> Three indices of cortisol status were compared with one another, namely, initial sample, the relative immobilisation stress response and the total adrenal response.

a) <u>Initial sample</u> - was represented by the first blood sample collected following immobilisation, irrespective of the elapsed time after darting. This sample would closely represent the unstimulated basal level as controlled experiments have shown that the loss of consciousness brought about by dissassociative anaesthetics represents the stressor and not the darting (Sapolsky 1982).

b) <u>Relative immobilisation stress response</u> - was calculated as the maximum increase recorded during the 90 min sampling period following immobilisation. This maximum response was expressed as a percentage of the initial sample.

c) <u>Total adrenal response</u> - was calculated as the area beneath the curve from the time of darting (0) for a period of 90 min.

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Results

<u>Cortisol assay validation</u>: Antiserum specificity was determined by the suppliers (BioMakor). Cross reaction with other steroids were as follows: compound S 13,6%, corticosterone 11,7%, deoxycorticosterone 6,0%, 17α-OH-progesterone 9,4%, progesterone 6,9%, aldosterone 0,5%, androstenedione 0,5%, testosterone 4,0%, dehydroepiandrosterone, oestradiol and dexamethane < 0,01%, prednisolone < 1,0%. Sensitivity of the assays ranged from 0,4 - 3,2 ng/ml ($\bar{x}$ = 1,6 $\pm$ 1,2 s.d.; n = 6). Extraction efficiency ranged from 81,72 - 95,30% ($\bar{x}$ = 89,34; n = 6) and was unaffected by plasma volume (50 - 200 μl). Results were corrected accordingly. Buffer blanks contained 2,99 $\pm$ 4,29 s.d. ng cortisol equiv./ ml (n = 6). The addition of cortisol (50, 100, 200 pg) to a plasma pool resulted in recoveries that did not differ significantly ($t_{0,05[4]}$ = 0,17; P > 0,5) from expected values, and parallelism was demonstrated over the entire range of the standard curve. Intra- and inter-assay coefficients of variation were 6,7% and 10,2% respectively.

<u>Cortisol status</u>: The recorded cortisol responses (0 - 90 min) following immobilisation for individual females and males are shown in Fig. 11. Those categories that provide the highest resolution for each sex , namely the reproductive categories for females and the social categories for males were used to group the responses. An analysis of the relationships between the various indices of cortisol status are given in Table 3.

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TABLE 3. Calculated relationships between the three cortisol parameter estimates in the spotted hyaena ($n = 22$). All estimates were collected from the same animals during a single immobilisation. SAS PROC CORR SPEARMAN (r_S).

Parameter estimates	Spearman's coefficient (r_S)	Significance level
1. Initial sample *vs* Total response	0,91	P < 0,001
2. Initial sample *vs* Relative response	-0,51	P < 0,05
3. Total response *vs* Relative response	-0,33	P > 0,10

These results indicate a strong positive relationship between the initial sample and the total adrenal response. On the other hand, a significant negative relationship was recorded between the initial sample and the relative immobilisation stress response, suggesting an inverse relationship. Although there was no relationship between the total adrenal response and the relative immobilisation stress a negative tendency was evident (Table 3).

Visual inspection of the cortisol profiles (Fig. 11) fails to show any clear pattern, however, the following was evident: first, a large variance in the responses was recorded within each reproductive and social category,

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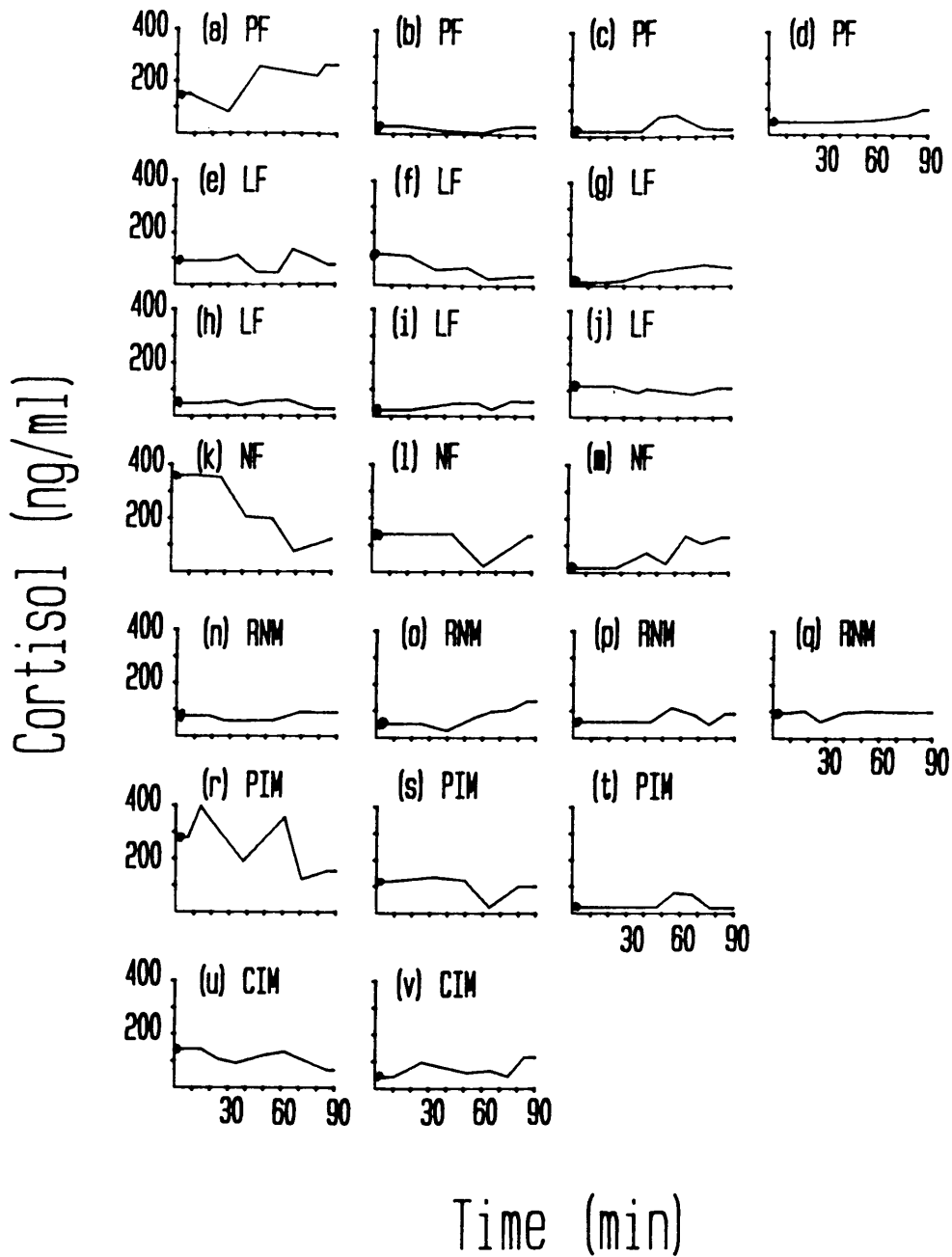Fig. 11. Cortisol profiles for 13 female and 9 male spotted hyaenas anaesthetised with Halothane following Zoletil immobilisation. Two cortisol status parameter estimates, namely, initial sample (●) and the total adrenal response (0 to 90 min) are indicted. PF = parous female, LF = lactating female, NF = nulliparous female, RNM = resident natal male, PIM = peripheral immigrant male, and CIM = central immigrant male.

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and the fluctuations within individual responses varied extensively. There was no response recorded in most individuals (55%), whereas a number of animals showed declining cortisol titres (18%) following immobilisation. Seemingly positive responses were recorded in 27% (Fig. 11 a,d,g,m,o,v) of the animals subjected to serial sampling after immobilisation. The highest total responses, exceeding values of 200 ng/ml, were found in three individuals, namely a parous female, a peripheral immigrant male and a nulliparous female.

The strong correlation between the initial sample and the total adrenal response does, however, enable a statistical analysis of changes in plasma cortisol concentrations during the socio-ecological development of this species. Analyses of initial cortisol titres showed no relationship with age, and the mean plasma cortisol concentrations did not vary among the sexes [$\bar{x}$ = 60,88 $\pm$ 14,57 s.e.m. for females (n = 26) $cf.$ $\bar{x}$ = 59,35 $\pm$ 10,16 s.e.m. (n = 33) for males]. Parametric analyses of variance (cortisol^{0,05}) failed to reveal any significant differences between the various reproductive (PROC GLM: $F_{0,05[4,58]}$ = 1,33; P > 0,05) and social categories (PROC GLM: $F_{0,05[5,56]}$ = 2,04; P > 0,05) - (see Fig. 12). However, the mean cortisol concentrations showed that MMs and NFs were elevated among the reproductive categories, whereas MCs and FCs had lower, and PIMs had higher mean cortisol concentrations than the other social categories. An interesting additional trend is the relatively high mean plasma cortisol concentration ($\bar{x}$ = 133,90 $\pm$ 71,41 s.e.m; n = 5) found in those females in the age group between weaning and puberty ($\pm$ 1 - 3 yrs). This trend is illustrated by the relatively high mean plasma cortisol concentrations in nulliparous females (Fig. 12a) that decline drastically when the female cubs are grouped on their own (Fig. 12b).

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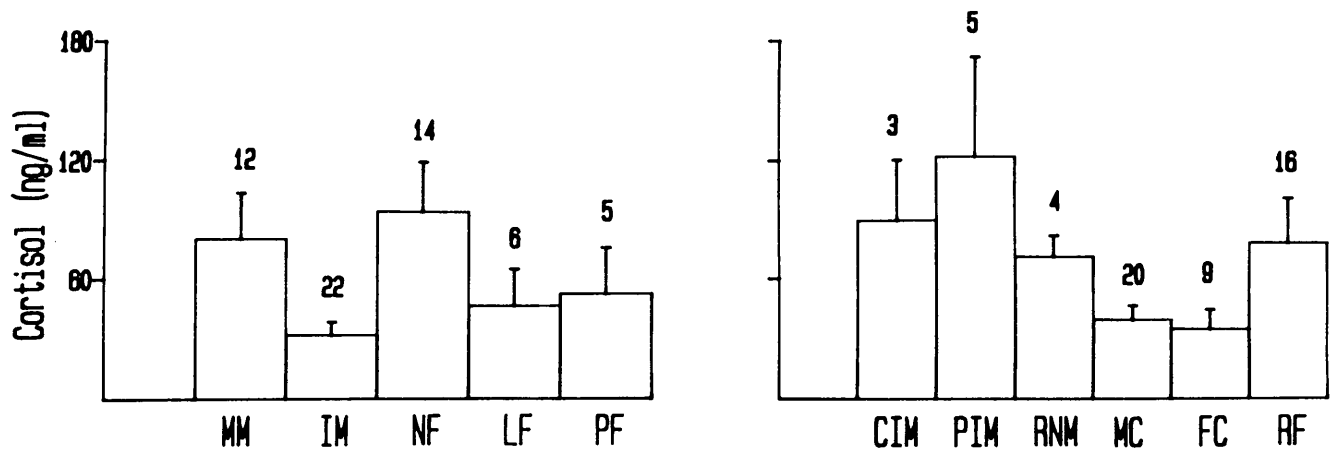
Fig. 12. Mean ($\pm$ s.e.m.) peripheral cortisol concentrations for:

(a) <u>Reproductive categories</u>: MM = mature males (> 2 yrs), IM = immature males (< 2 yrs), PF = parous females, LF = lactating females, NF = nulliparous females, and

(b) <u>Social categories</u>: CIM = central immigrant males, PIM = peripheral immigrant males, RNM = resident natal males, MC = male cubs, FC = female cubs, RF = resident females. ANOVA was run on (cortisol)^{0,05}. Statistical differences (*) were tested at P < 0,05.

The relative cortisol responses to immobilisation stress differed markedly from those patterns observed using the initial samples and the total adrenal responses as indices of cortisol status. The expression of the relative cortisol responses in a statistical manner yields a data spread that could easily be taken to represent a mean positive response over time for the 22 animals subjected to serial sampling after immobilisation (see Fig. 13). However, closer scrutiny of the individual responses reveals that all those responses over 200% of the initial sample can be attributed to only three females, namely, a parous, lactating and nulliparous female.

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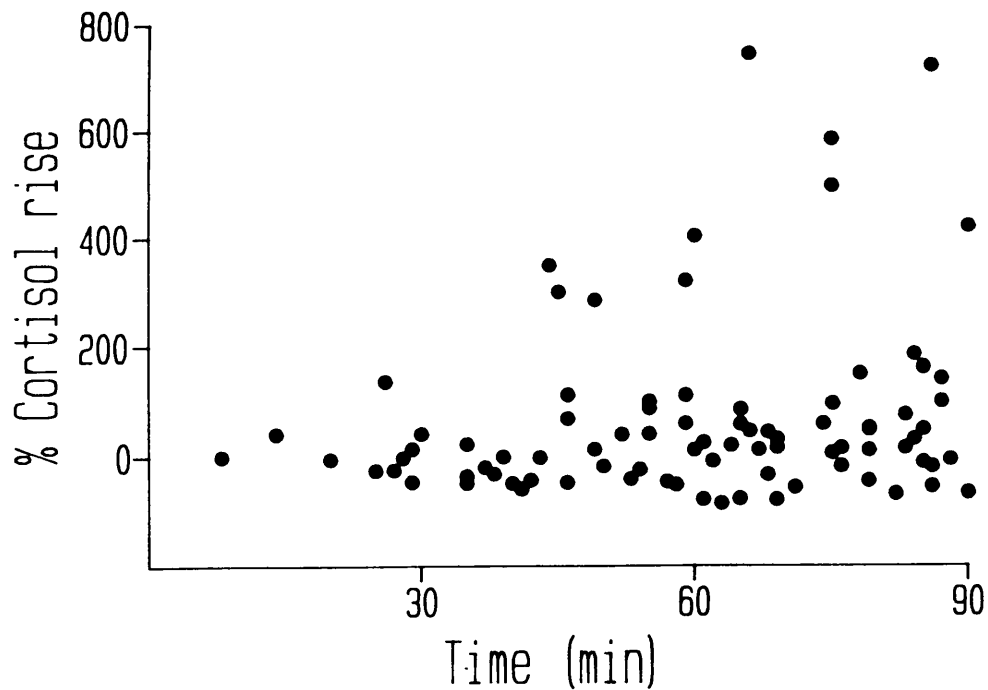
Fig. 13. Relative cortisol responses over time in 22 spotted hyaenas subjected to immobilisation stress (0 - 90 min). Initial immobilisation was with Zoletil and the animals were maintained using Halothane.

In an attempt to explain the observed variance in cortisol responses within the various social and reproductive categories, the existance of correlations between initial cortisol titres and other circulating steroids were investigated. In females there was a negative relationship between plasma cortisol and androstenedione (r = -0,52; P < 0,05; n = 16). A similar analysis of the relationship between the relative cortisol response and other steroids for those categories with a sufficient sample size, revealed that both testosterone (r = 0,91; P < 0,02; n = 6) and androstenedione (r = 0,93; P < 0,01; n = 6) had a significant positive relationship. However, only the PIMs showed a positive correlation (r = 1,00; P < 0,05; n = 3) between relative cortisol responsiveness and testosterone for males.

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The observed plasma cortisol profiles from initial samples for male and female cubs are shown in Fig. 14. Although there appears to be a decline in mean initial cortisol levels recorded in cubs when compared with older individuals (Fig 12b), this did not reach significant statistical levels. In the female cubs basal cortisol levels seem depressed, with the highest cortisol concentration appearing in the four day old male cub (Fig. 14).
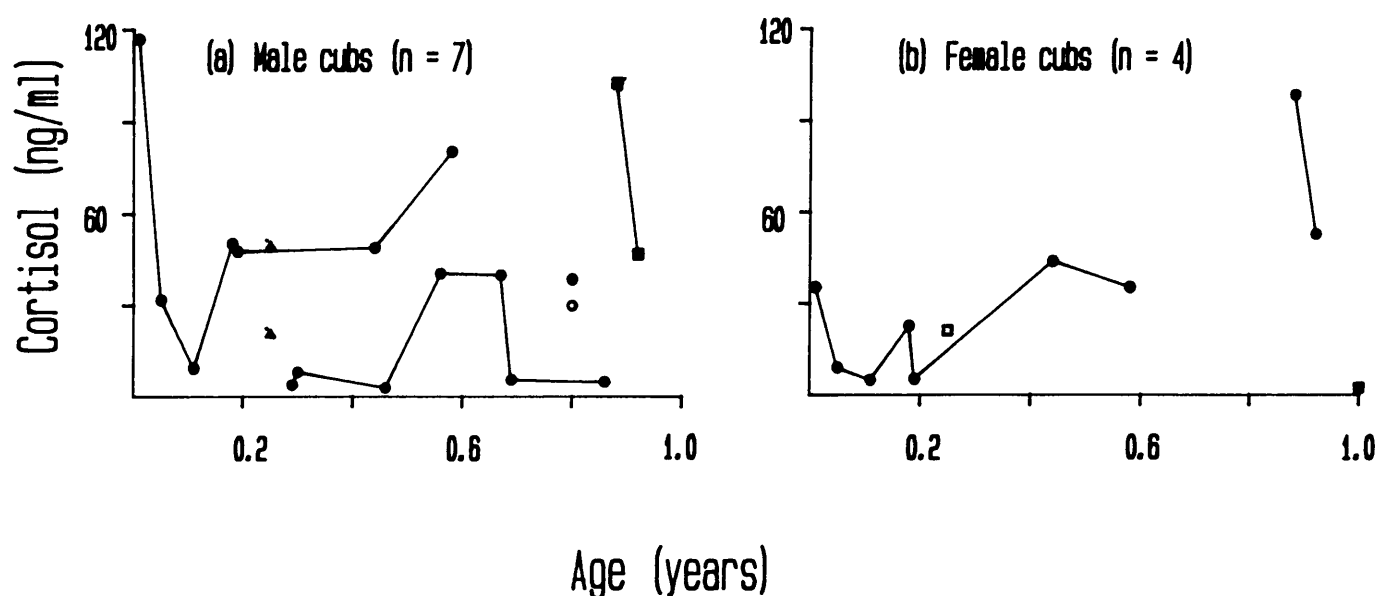
Fig. 14. Plasma cortisol concentration profiles for (a) male and (b) female cubs. Serial samples taken from the same animals are connected.

Discussion

The spotted hyaena social unit or clan comprises philopatric females, their cubs and three social classes of males: resident natal males, peripheral immigrant males and central immigrant males, representing the various stages of male biased dispersal tactics. The clan is organised in a near-

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linear social hierarchy with mature adult females being dominant, followed by other natal members, then central immigrant males and peripheral immigrant males (Frank 1986a, Henschel & Skinner 1987). This strict social hierarchy is established and maintained through agonistic interactions and plays an important role in establishing feeding priority and relative carcass division (Frank 1983, Tilson & Hamilton 1984, Henschel & Skinner 1987).

Plasma testosterone and androstenedione concentrations in males are largely governed by the dispersal stage of males (chapter 3) and only those males that had successfully dispersed and procured mating rites, namely the central immigrant males (CIMs) have elevated testosterone concentrations. This, together with the significant role played by social aggression in spotted hyaena society suggests that plasma cortisol titres may be indicative of social stressors encountered during the social development of this carnivore. Although similar endocrine processes are well documented in captive and wild primates (Sapolsky 1982, 1983a, 1983b, 1985, Martensz, Vellucci, Fuller, Everitt, Keverne & Herbert 1987) it appears that captivity and the method of artificial group formation may significantly affect results (see Yodyingyuad, Eberhart & Keverne 1982, Eberhart, Keverne & Meller 1983). Thus, the present study represents the first investigation of the relationship between adreno-cortical responsiveness and social development in an unmanipulated social carnivore.

Current theory from the rodent and primate literature suggests that higher ranking subjects should show a greater relative rise in cortisol concentrations in response to stress, but have lower basal cortisol concentrations (Louch & Higginbotham 1967, Manoque et al. 1975, Sapolsky

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1983b, McGuire, Bremmer & Raleigh 1986). Neither of these features were significantly evident in the spotted hyaena (Fig. 12), and although this analysis is based on crude dominance related social categories, the large variances recorded within each category are difficult to explain. Therefore, the principle feature of the results presented here is the heterogeneous nature of the cortisol responses observed. Females did however have a tendency to show a positive relative response, whereas those males subjected to social stress on a regular basis (PIMs) has slightly higher mean absolute responses. This could be indicative of a greater capacity to secrete cortisol in the PIMs, and a higher degree of stress responsiveness in females who are usually the dominant grouping (Kruuk 1972, Frank 1986a, Henschel & Skinner 1987). However, the inconsistent nature of these trends in this study means that the individual variability in stress-responsiveness fails to support the "social turmoil" hypothesis as a general mechanism. Possibly individual differences in stress responsiveness, such as recorded in olive baboons (Sapolsky & Ray 1989), could explain the recorded variance in adreno-cortical responsiveness.

The tendency towards lower basal plasma cortisol concentrations observed in both male and female cubs (Fig. 12b) could be indicative of some degree of stress non-responsiveness occurs during the post-natal development of the spotted hyaena. This proposed period of unperturbed low levels of cortisol is characterised by a reduced adrenocortical responsiveness to stress and reduced basal concentrations (Sapolsky & Meaney 1986). However, no significant decline could be demonstrated in this study. Earlier reports showed that androstenedione was of adrenal and ovarian origin in this species (Lindeque *et al.* 1986, Glickman *et al.* 1987), and that high concentrations of androstenedione were found in NFs and FCs with a

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subsequent decline in PFs (Glickman *et al.* 1987, present study). This and the negative correlation between plasma cortisol and androstenedione observed in female spotted hyaenas in the present study, suggests that the reduced cortisol responsiveness and the high plasma androstenedione concentrations may be related events. However, in the case of the spotted hyaena would imply that the paucity for adrenal cortisol genesis, which is probably the result of a unique pattern of glucocorticoid-receptor concentrations that exist in the brain and pituitary of the neonate (Sapolsky & Meaney 1986) extends over most of the first year of life. On the other hand, the sex specific capacity of FCs to secrete androstenedione from both the ovaries and adrenals lasts up to puberty (Chapter 3), and therefore, present understanding suggests that the androstenedione hypersecretion and cortisol hyposecretion are unrelated events. Naturally the physiological basis for increased androstenedione secretion in FCs requires further investigation.

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CHAPTER 6 - PROLACTIN STATUS

Introduction

Although prolactin is usually associated with milk production, it also stimulates other functions related to metabolism, growth, osmoregulation and reproduction in mammals (McNeilly 1987, Meijer, Trudeau, Colenbrander, Poot, Erkens & Van de Wiel 1988). Moreover, it seems to be directly involved in a number of physiological states characterised by reduced gonadotrophin secretion and impaired reproductive activity, including puberty, seasonal and lactational anoestrus as well as infertility resulting from stress in several species (Keverne 1979, McNeilly 1980, Haynes & Howles 1981, Eberhart *et al.* 1983, Mondain-Monval, Moller, Smith, McNeilly & Scholler 1985).

Since early work (Nicoll, Talwalker & Meites 1960, Grosvenor 1965) first identified prolactin as a stress-responsive hormone, several studies have illustrated its responsiveness to induced pharmacological stressors (Quadri, Pierson & Spies 1978, Meller, Keverne & Herbert 1980, Aidara, Tahiri-Zagret & Robyn 1981), restraint procedures (Puri, Puri & Anand Kumar 1981) and social stressors such as subordination (Eberhart *et al.* 1983, Eberhart, Yodyingyuad & Keverne 1985). Changes in plasma prolactin concentrations during the socio-ecological development of the spotted hyaena were included in this investigation as the consensus of opinion is that, although prolactin responds to similar stressors as corticosteroids it seems to be under a different regulatory control mechanism (Naylor, Porter & Lincoln 1990).

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Methods

<u>Prolactin assay</u>: Spotted hyaena serum prolactin was determined using a homologous double-antibody radioimmunoassay kit for humans (IM. 1061; Amersham Laboratories, Buckinghamshire, England), as spotted hyaena prolactin standards or pituitary glands were unavailable. The assay uses ovine anti-human prolactin anti-serum, human pituitary standards (0, 5, 15, 50, 100 and 200 ng/ml) in human serum, ^{125}I human prolactin and donkey anti-sheep gamma globulin as second antibody. All freeze-dried constituents were reconstituted to specified volumes using glass distilled water. The incubation mixture consisted of reference preparations (100 μl) or serum samples (200 μl), antiserum (100 μl) diluted to 5,5 ml and ^{125}I-labelled human prolactin ($\sim$ 10 000 c.p.m. in 100 μl). The mixture was then incubated at room temperature ($\pm$ 20°C) for 24 h. Donkey anti-human gamma globulin (1 ml - second antibody) was then added and incubation continued at room temperature for 5 min before separation of bound and free hormone by centrifugation at room temperature (1500 g for 15 min). The free iodinated prolactin in the supernatant was decanted into scintillation vials and 4 ml scintillation fluid (Scintillator 299^{TM} ; Packard Instrument Co., Illinois USA) was added. Radioactivity was measured 4 h later for 2 min, using a Packard 1500 Tri-Carb scintillation counter (Packard Instrument Co., Illinois, USA). As no spotted hyaena standards were available the prolactin values were expressed in ng human prolactin equivalents/ml serum. Mathematical interpolation of recorded sample values against a standard curve was carried out using Securia^{TM} Plus RIA/QC software (Packard Instrument Co., Downers Grove, UK) over the range 5 - 200 ng/ml. The original volume serum included in the assay was taken into account when calculating the concentrations of prolactin in serum samples.

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Prolactin status: Two indices of prolactin status were evaluated namely the initial sample irrespective of the time since immobilisation, and the total prolactin response, represented by the area beneath the prolactin response curve from the time of immoblisation for a period of 90 min.

Results

Validation of the prolactin assay

Specificity. The method proved to be specific for spotted hyaena prolactin. The binding of ^{125}I-labelled prolactin to the antiserum was displaced in a parallel manner by serial dilutions of serum from a mature male and a lactating female (Fig. 15a). The addition of 200 μl serum from a male treated with bromocriptine to the standard curve did not affect the binding (Fig. 15a) and all samples were therefore assayed against the human standards. Measured prolactin concentrations in serum were not affected by volume (100-200 μl). The injection of LH-RH had no significant effect on serum prolactin concentrations (Fig. 15b), while androstenedione, the principle steroid secreted from the ovary following LH-RH stimulation (chapter 3) showed a marked positive response (Fig. 15c).

Accuracy. The recovery of known amounts of human prolactin (15 - 100 ng) added to the serum of a spotted hyaena treated with bromocriptine did not differ significantly from expected values ($t_{0,05[2]}$ =1,81; P > 0,2).

Sensitivity and precision. Sensitivity was 0,76 $\pm$ 0,23 ng/ml serum. The proportion of total radioactivity bound by the anti-serum in the absence of hormone ranged from 48,38 to 54,04 %. The intra- and inter assay coefficients of variation were 2,04 % and 14,12 % respectively.

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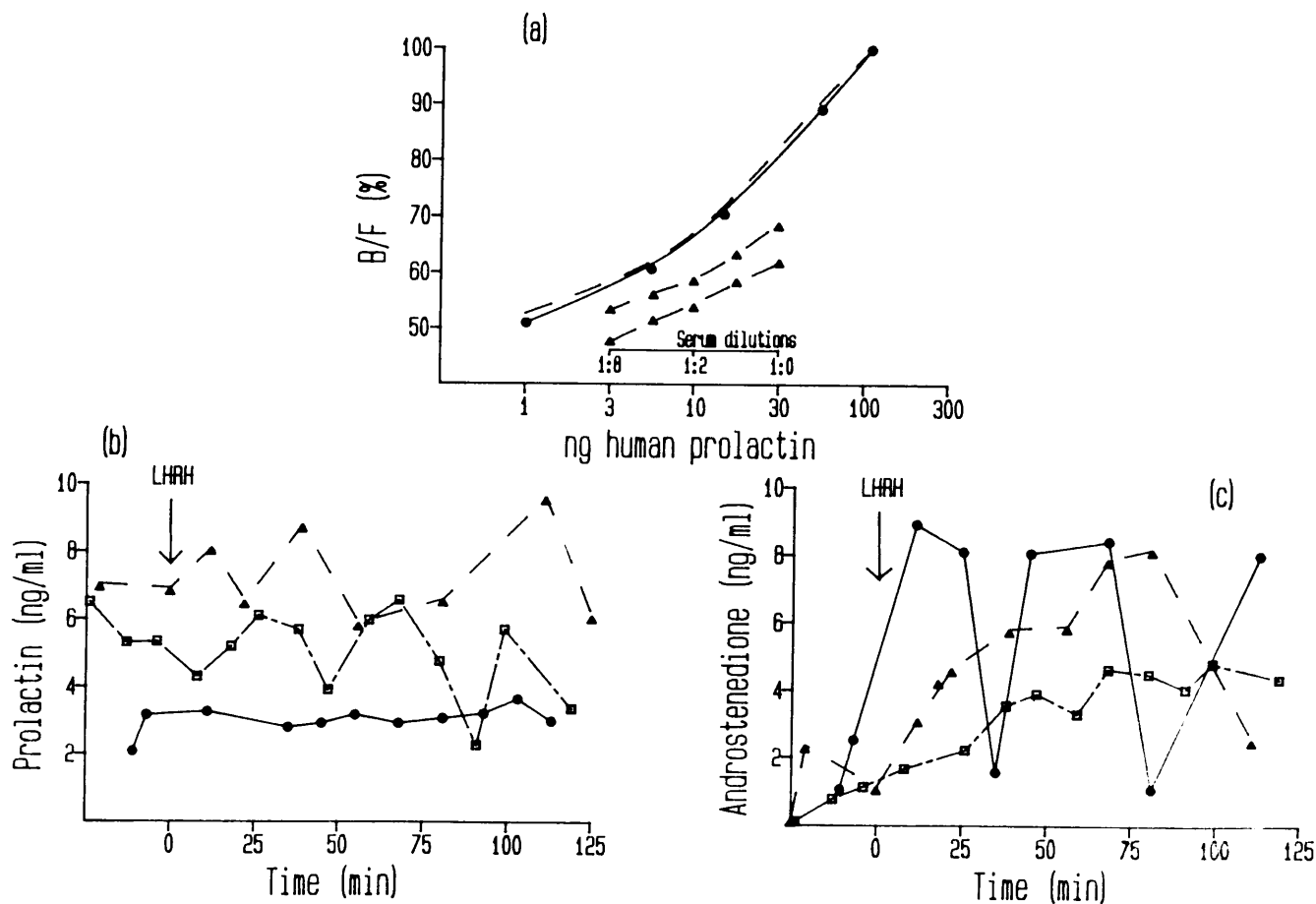
Fig. 15. (a) Standard curve for human prolactin (Amersham: IM 1061) prepared in distilled water with (- - - -) and without (———) 200 µl serum from a male spotted hyaena treated with bromocriptine, and dose response curves for dilutions of spotted hyaena serum. (b) Serum prolactin concentrations after an injection of 1 µg/kg LH-RH (LH-RH: Hoechst), and (c) androstenedione responses from the ovary following the injection of 1 µg/kg LH-RH.

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Biological validation. All the treated spotted hyaenas showed increased serum prolactin concentrations after the injection of metoclopramide, the effect being significantly greater in the immature male than in a nulliparous female and a mature male. A decrease was seen after the injection of bromocriptine (Fig. 16).
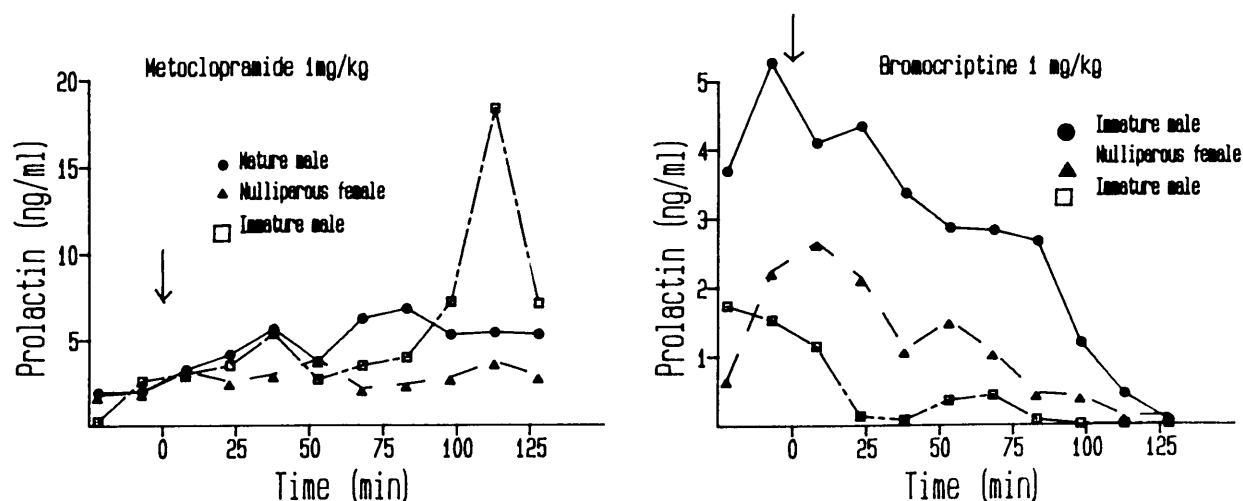
Fig. 16. Serum prolactin concentrations in male and female spotted hyaenas after the injection (↓) of metoclopramide or bromocriptine.

Prolactin status: Correlation analysis of the relationship between the two indices of prolactin status showed that there was a strong positive rank correlation (r_s = 0,78; P < 0,001; n = 22) between the initial sample and the integrated prolactin response for 90 min. The profiles obtained from the animals subjected to serial sampling are given in Fig. 17. In twelve (55 %) of the animals the highest prolactin concentrations recorded were the initial samples. Of the ten animals that showed subsequent increases, six (27 %) were represented by the lactating females in the sample, all of which tended to show a general increase after starting from low initial values (Fig. 17 e-j). Other animals that showed subsequent increases in serum prolactin concentrations after the initial sample included two RNMs

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(Fig. 17 o,q), one PIM (Fig. 17 r) and a PF (Fig. 17 c). This tendency to increase and decrease after first sampling showed no relationship with the time of first sampling, as those individuals that showed a relative increase were on average first sampled after those that showed a decline ($\bar{x}$ = 18,90 min cf $\bar{x}$ = 3,82 min). Visual inspection of the prolactin profiles also shows that males generally showed a larger variance in prolactin concentrations following immobilisation stress than females.
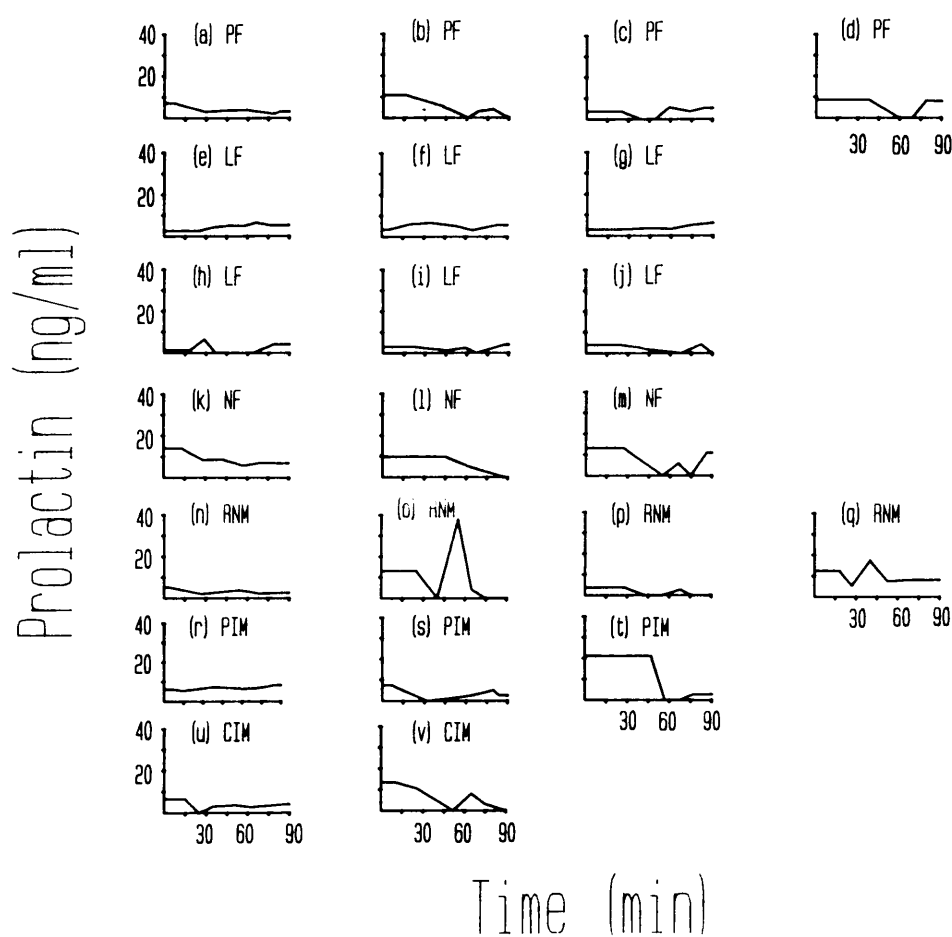
Fig. 17. Serum prolactin profiles from 22 animals immobilised with Zoletil and maintained for 90 min on Halothane. PF = parous female, LF = lactating female, NF = nulliparous female, RNM = resident natal males, PIM = peripheral immigrant males, and CIM = central immigrant males.

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Using the initial values as an index of prolactin status, a parametric ANOVA (PROC GLM) comparing $\log_{10}$(prolactin) concentrations between sexes failed to show any significant difference $(F_{0,05[1,55]} = 4,6)$. A similar analysis for the various reproductive $(F_{0,05[4,55]} = 15,79)$ and social $(F_{0,05[5,53]} = 3,65)$ categories showed significant variance. A comparison of means revealed that LFs had significantly lower $(HSD_{51} = 3,99; P < 0,05)$ prolactin concentrations than all the other reproductive categories, while PFs showed a lower mean prolactin concentration than the remaining categories without attaining statistical significance.
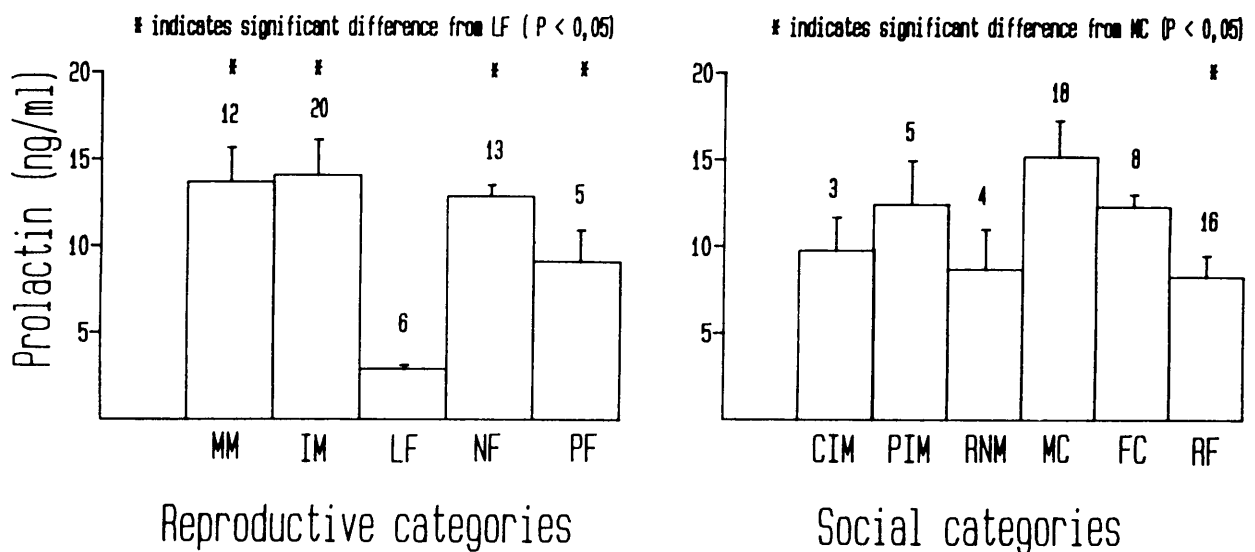
Fig. 18. Mean ($\pm$ s.e.m.) serum human prolactin equiv. for:

(a) Reproductive categories: MM = mature male (> 2 yrs), IM = immature males ($\leq$ 2 yrs), PF = parous females, LF = lactating females, NF = nulliparous females, and

(b) Social categories: CIM = central immigrant males, PIM = peripheral immigrant males, RNM = resident natal males, MC = male cubs, FC = female cubs, and RF = resident females. ANOVA was run on $\log_{10}$(prolactin). Statistical significance was measured at P < 0,05.

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Among the social categories the PIMs, MCs and FCs had elevated mean prolactin concentrations. However, these did not reach statistical significance, and only the MCs were found to have significantly higher $(HSD_{48} = 4,197; P < 0,05)$ titres than RFs.
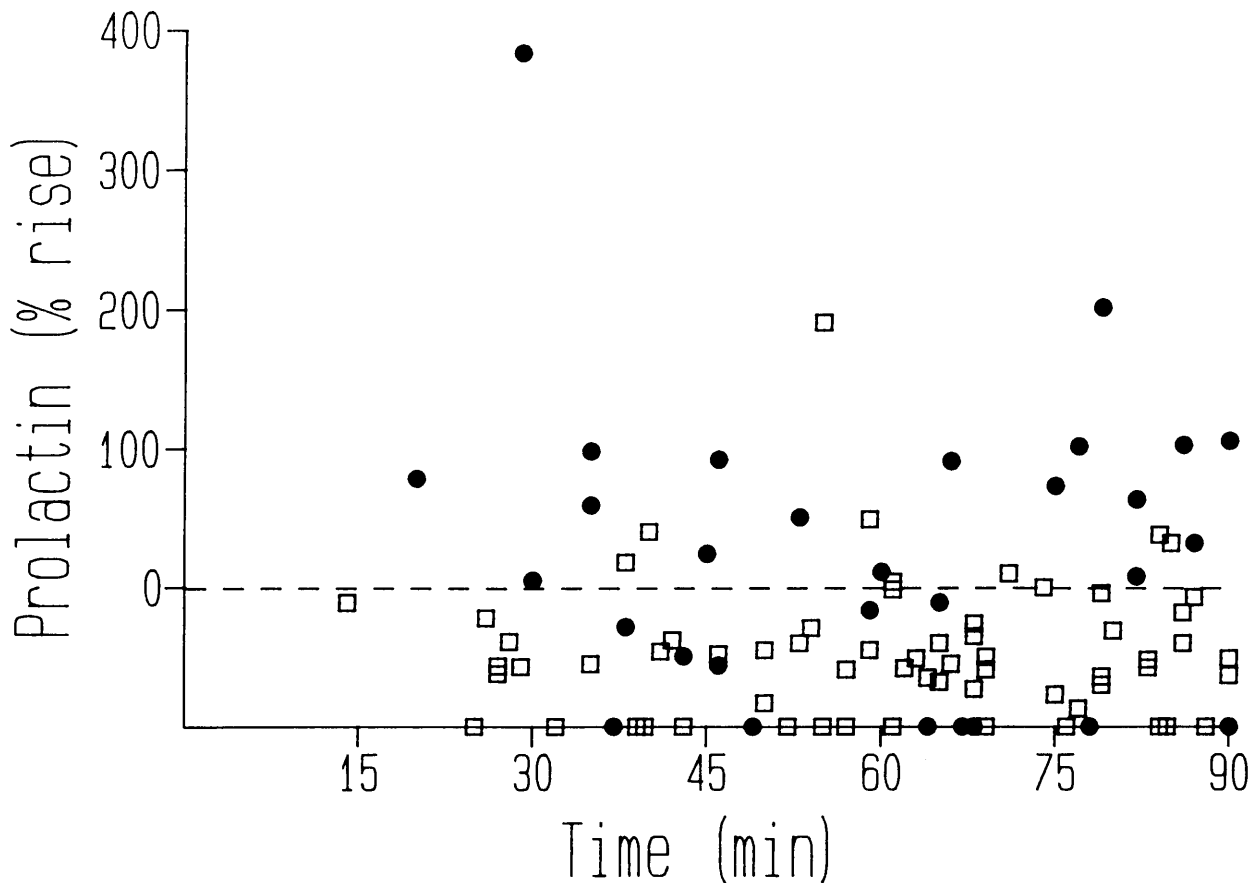
Fig. 19. Relative prolactin responses over time in 22 spotted hyaenas subjected to immobilisation stress (0 - 90 min); ● = females and □ = males. Initial immobilisation was with Zoletil and the animals were maintained using Halothane.

The expression of the changes in plasma prolactin concentration after immobilisation stress as a percentage of the initial value (Fig. 19) illustrates that most animals show a decline in peripheral prolactin

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concentrations following immobilisation. However, some do increase their prolactin titres, especially the lactating females. Again, as was evident from the profiles (Fig. 17) only three males and a single parous female showed increases above the level of the initial sample, besides the large number of lactating females that tended to increase prolactin concentrations after the initial sample.

Furthermore, there was no significant difference between male and female cubs in either their mean plasma prolactin concentrations (Fig. 18b), or in the observed trends during the first year of development (Fig. 20). More important, however, is that there was no sign of reduced prolactin titres during this developmental phase. On the contrary there seems to be a tendency to increase prolactin titres during this developmental stage (Fig. 18b), although the apparent increase did not reach statistical significance.
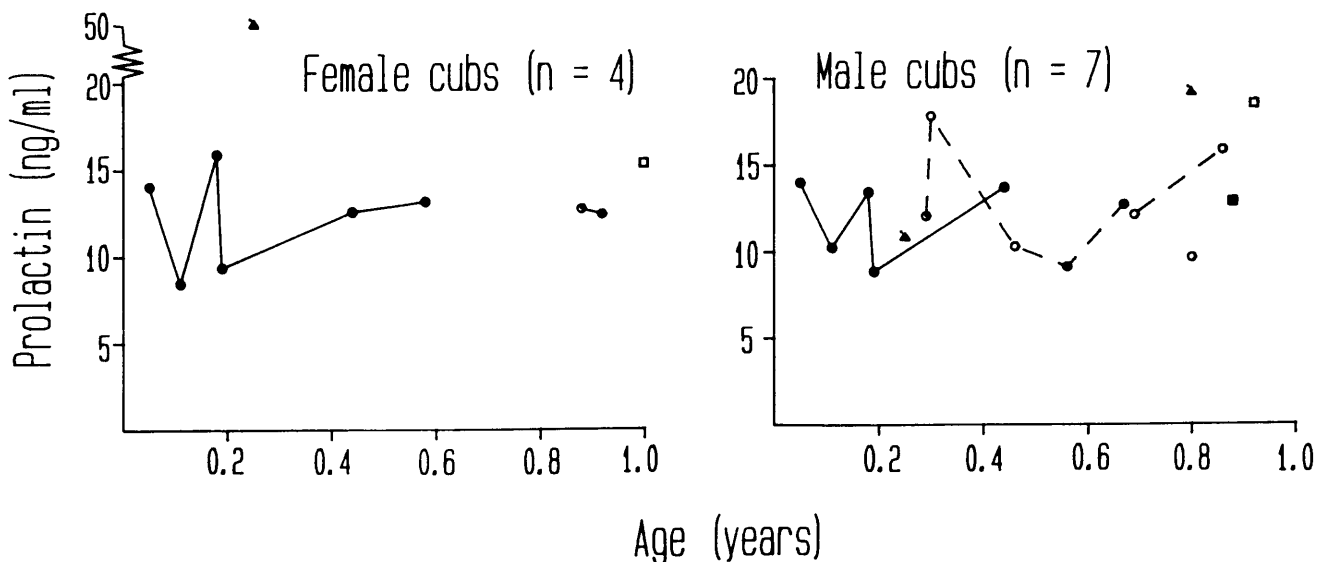
Fig. 20. Serum prolactin profiles for (a) female and (b) male cubs. Serial samples from the same animals are connected.

In order to investigate the relationships between prolactin responsiveness, the concentrations of steroid hormones and age, correlation analyses were executed. A significant negative relationship (r = -0,28; P < 0,05; n = 56) between age and initial prolactin concentrations was observed, reflecting the higher prolactin responsiveness in cubs observed above (Fig. 18b). Positive correlations were also observed between plasma prolactin concentrations and androstenedione (r = 0,79; P < 0,001; n = 17) in MCs, and prolactin and testosterone (r = 0,96; P < 0,05; n = 4) in RNMs. Negative correlations were observed between plasma prolactin and cortisol in MMs (r = -0,59; P < 0,05; n = 12), and for the whole sample between prolactin and progesterone (r = -0,30; P < 0,05; n = 50), and oestradiol-17ß (r = -0,29; P < 0,05; n = 50) respectively. A strong negative correlation between plasma prolactin and progesterone concentrations was found in the LFs (r = -0,87; P < 0,05; n = 6).

Discussion

Although it is generally accepted that serum prolactin concentrations are increased during acute stress, this is not always the case, as a decrease in serum prolactin concentrations often occurs when concentrations have already been raised (Krieg, Lamberts & MacLeod 1984, Gala & Haisenleder 1986) or during chronic stress (Collu, Tache & Ducharme 1979, López-Calderon, Ariznavaretta, Calderón, Tresguerres & Gonzalez-Quijano 1989). Thus, the general trend of declining serum prolactin concentrations observed in animals subjected to serial sampling in this study suggests that some degree of prolactin stress responsiveness to immobilisation stress had already occurred by the time of first sampling. The mean time of first sampling for this serial sample was 20,23 min and rats are known to

show increased levels after 20 - 45 min following immobilisation stress (López-Calderón et al. 1989). The initial samples used in the present study would therefore represent stressed values and not basal levels.

The lack of any significant differences in prolactin stress responsiveness recorded in this study (Fig. 18) follows earlier work on captive talapoin monkeys that found no clear correlation between male social rank and prolactin concentrations (Yodyingyuad et al. 1982), and concluded that factors such as socio-sexual interactions (Eberhardt et al. 1983) and the duration of subordinance (Eberhardt et al. 1985) may affect the way that prolactin concentrations change, as well as the relationship of prolactin with that of cortisol. However, the mean prolactin changes show a tendency to reflect the social stressors associated with the male dispersal pattern of wild spotted hyaenas, which implicitly includes certain socio-sexual and dominance related aspects.

The negative correlation observed between plasma prolactin and cortisol may also be indicative of negative feedback between adrenocortical activity and the prolactin stress response, a process documented in the male rat (López-Calderón et al. 1989). On the other hand, it does not support the suggested role played by prolactin in counteracting the harmfull effects of glucocorticoid secretion (Drago, Agata, Iacona, Spadaro, Grassi, Valerio, Raffaele, Astuto, Lauria & Vitetta 1989).

The mean prolactin concentration found in the PIMs is similar to that observed in the prepubertal MCs and FCs (Fig. 18b), as well as prepubertal pigs (Sus scrofa) and seasonal breeding blue foxes (Alopex lagopus) - (Mondain-Monval et al. 1985, Smith, Mondain-Monval, Moller, Scholler &

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Hansson 1985). These higher mean prolactin concentrations suggest that the relative testicular inactivity (chapter 3) of dispersing males (PIMs) and gonadal inactivity of prepubertal spotted hyaenas may, in certain cases , be associated with hyperprolactinaemic conditions (McNeilly 1980, Haynes & Howles 1981). Moreover, the tendency for mean serum prolactin titres to decline in the RNMs between puberty and dispersion is accompanied by a transition from the prepubertal to pubertal testis. This is suggested by the switch in a positive correlation between serum prolactin and androstenedione in MCs, to a positive correlation between prolactin and testosterone in RNMs. An aspect of spotted hyaena endocrinology that remains to be elucidated is how female cubs manage to secrete large quantities of androstenedione (chapter 3) while the adrenal may show reduced cortisol responsiveness (chapter 4) and the animal appears to be hyperprolactinaemic.

Among the reproductive categories lactating spotted hyaenas are prominent for their low serum prolactin concentrations (Fig. 18a). These low prolactin concentrations recorded during lactation follow the pattern previously observed in the rabbit (*Oryctolagus cunniculus*) and the brown hare (*Lepus europaeus*) - (McNeilly & Friesen 1978, Caillol, Mondain-Monval & McNeilly 1990). In contrast prolactin concentrations are elevated for the entire duration of lactation in cats (*Felis catus*)-(Banks, Paape, & Stabenfeldt 1983) and blue foxes (Mondain-Monval *et al.* 1985). This species specific difference has been attributed to differences in suckling patterns where rabbits and hares suckle their young only once a day compared to the frequent suckling observed in cats (Caillol *et al.* 1990). Similarly, although spotted hyaena cubs are dependent on milk for 4-6 months and are not weaned until 14-18 months of age, the lactating females are often

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forced to move large distances in search of food, and consequently, mothers may be away from their cubs for up to five days (Kruuk 1972, East, Hoffer & Turk 1989). Moreover, these reduced prolactin concentrations observed during lactation are in agreement with previous reports concerning the lack of a distinct lactational anoestrus in this polyoestrus aseasonal breeder (Lindeque 1981, Lindeque & Skinner 1982b, Lindeque *et al.* 1986).

The negative correlations recorded between plasma prolactin and progesterone concentrations in lactating females suggests that, similar to the rat (Deis, Leguizamon & Jahn 1989), a feedback regulation of stress-induced prolactin release by progesterone may exist in the spotted hyaena. Furthermore, the negative correlations observed between plasma prolactin concentrations and both progesterone and oestradiol-17ß for the sample as a whole, together with the recorded positive correlation between plasma progesterone and oestradiol-17ß in females (chapter 4), suggests that prolactin secretion declines when ovarian activity increases. This relationship, together with the demonstration of significant follicular activity during lactation (Lindeque 1981) could explain the absence of a distinct lactational anoestrus in this species.

CHAPTER 7 - ENDOCRINE VARIANCE AND SOCIO-ECOLOGICAL DEVELOPMENT

"Natural science does not simply describe and explain nature; it is a part of the interplay between nature and ourselves; it describes nature as exposed to our method of questioning" .... (Heisenberg 1958)

Introduction

One of the principle aims of scientific enquiry is to explain extant phenomena in a manner which would facilitate the making of predictive statements about the future. However, in this pursuit the various scientific disciplines are confronted by different limitations. The material of the physicist lends itself, as a rule, to displays of regularity called "laws of nature" that can be quantitatively expressed. The discovery of such regularities requires the existance of *homogeneous* classes in nature that can be identified using repetitive experiments. The existance of such classes that consist of homogeneous entities (i.e. the class of electrons) then allows the quantitative description of a class normality (μ) together with a variance (σ). Moreover, in their efforts to understand more complex phenomena scientists usually adopt the Cartesian approach (Descartes 1637), in which complex entities are broken down into smaller configurations which are more likely to be homogeneous. An analysis of the functions of these homogeneous configurations then allows one to put the object under investigation back together again, at least mentally, and to arrive at some understanding of the working of a complex structure.

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In contrast to the inorganic world, biological entities are not characterised by the existance of homogeneous classes but by the existance of *heterogeneous* classes (Elsasser 1981). A heterogeneous class implies that all members of such a class can be distinguished from other individuals using an appropriate level of enquiry. The existance of heterogeneous classes in biology means that biology is essentially a "non-Cartesian" science (Elsasser 1981, Elsassar 1984), and that biological complexity cannot be <u>fully</u> understood using the concept of class normality (μ). This lack of a meaningful normalcy in biology means that the observed variance (σ) is a reality and should not simply be ascribed to differential boundary conditions or measurement errors. In essence, biology should be a science directed towards the description of the nature of biological variety. The heterogeneous nature of biology can also be viewed as the result of a selection process from among many possibilities (Elsasser 1981), implying that the living forms that we observe are discrete points on a continuum of organisational possibilities. Biological variety therefore exists because biological systems are very sensitive to initial conditions (Jensen 1987) and only certain permutations are viable under particular conditions. This view in effect gives rise to the intriguing possibility that the central question in biology is not: why is there so much biological variety ?, but rather, why is there so little variety ? (Pollard 1984), and also gives some substance to the Darwinian (Darwin 1859) notion of "natural selection". Thus, the principle task facing biologists is to determine the nature of this process and to define appropriate quantitative indices of emergent biological variability.

With these views in mind, our attention will now return to the central theme of this thesis, which is to investigate the endocrine correlates of social development in the spotted hyaena. Most of the work in the earlier chapters was aimed at describing changes in mean endocrine parameters during the socio-ecological development of this species. In this chapter the aim will be to investigate whether the description of hormonal variances may bring additional insights to the endocrine dynamics of this species. That variances in endocrine patterns exist in the numerous stages of the socio-ecological development of this species was established in the preceding chapters. However, the large number of variables involved make it difficult to form any comprehensive picture of how these variances interact. In order to facilitate the identification of important underlying trends and patterns, multivariate analysis procedures were employed on the normalised ($\log_{10}$ transformation) hormonal data set.

Methods

MANOVA. Multiple analysis of variance (MANOVA) was employed to establish a comprehensive picture of the interactions between the means of the measured hormonal variables. Mean hormone concentrations among the various reproductive and social categories were compared to one another, and were used to test for the presence of a significant combined effect of the reproductive and social categories. The MANOVA was run using the SAS GLM procedure.

Principle component analysis (PCA). PCA was run on the sample as a whole to establish both the nature of the principle axes of variance for the hormonal hypervolume, and to identify any outliers in the data set. In

addition PCA effectively reduces dimensionality and therefore simplifies the interpretation and explanation of underlying patterns. PCA was run using the SAS PRINCOMP procedure, first on the complete data set and then on males and females separately.

Results

MANOVA. Significant differences in mean values were obtained within the reproductive categories ($F_{[15,97]}$ = 7,8; P < 0,001) and the social categories ($F_{[20,117]}$ = 2,6; P < 0,001). The various Duncan groupings (those groups that do not differ significantly from one another) are depicted in Fig. 21. There are a number of prominent features that emerge from the analysis. Cortisol showed no ability to differentiate between the various reproductive and social categories using a comparison of mean values. On the other hand, the CIMs were prominent among the social categories in terms of having significantly higher testosterone concentrations than all the other categories (Fig. 21 b). Likewise, both PFs and LFs among the reproductive categories (Fig. 21 a), differed significantly from all other categories, for the hormones oestradiol-17ß and prolactin respectively. Moreover, the categories originally described as social and reproductive categories, showed no combined effect on the mean hormonal parameter estimates. This means that their effects could be evaluated separately.

Principal component analysis (PCA). The results obtained for the PCAs performed in this investigation are given in Table 4. The first principal component (PC) of the complete sample consists of a strong total androgen effect, with both testosterone and androstenedione making large

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contributions to the total variance along this axis (see eigenvectors Table 4). The second PC contrasts high testosterone and cortisol concentrations
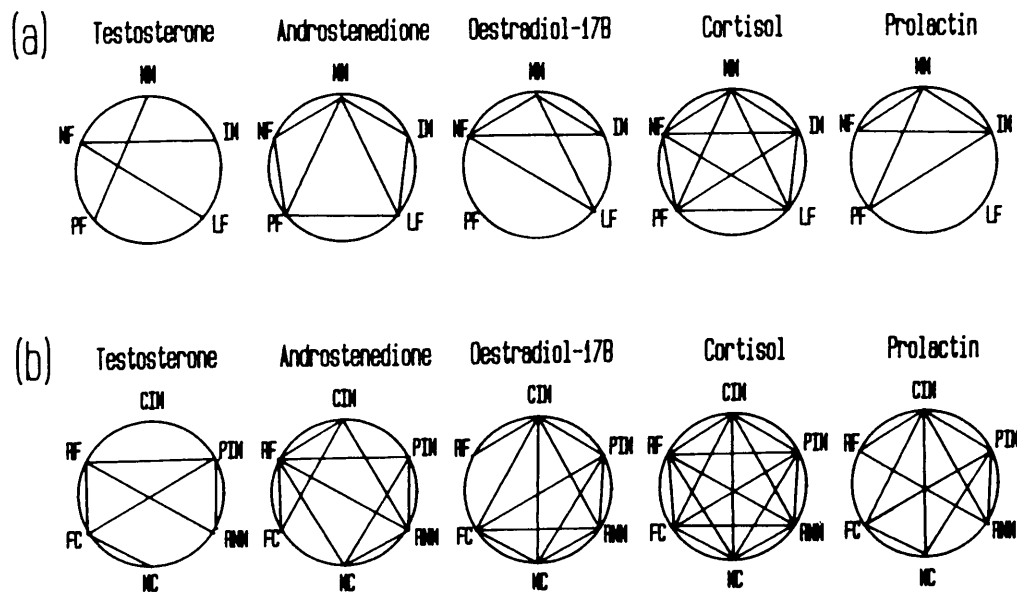
Fig. 21. Results of the MANOVA which tested for significant differences in mean values among (a) reproductive categories, and (b) social categories. The compared variables are depicted on the periphery of the circle, while connected variables indicate non-significant differences (P > 0,05) between categories.

against androstenedione. This in effect means that after the total androgen effect had been accounted for (PC-1) the main source of variation is between individuals with high testosterone and cortisol concentrations when compared with androstenedione concentrations. The third PC is a strong contrast between cortisol and testosterone, suggesting that when the variation from the first two PCs had been accounted for the biggest

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additional source of variance is between those individuals that have high cortisol concentrations compared with testosterone.

Table 4. Eigenvectors and eigenvalues for three PCAs performed on the complete hormonal hypervolume and the contributions of each to the total variation.

Variables (ng/ml x $\log_{10}$)	Total sample Eigenvectors PC-1	PC-2	PC-3	Females Eigenvectors PC-1	PC-2	PC-3	Males Eigenvectors PC-1	PC-2	PC-3
Testosterone	0,61	0,62	-0,46	-0,16	0,74	0,08	0,70	-0,71	-0,06
Androstenedione	0,73	-0,65	0,14	0,77	0,16	0,47	0,63	0,66	-0,38
Oestradiol-17ß	0,23	0,05	0,01	-0,10	0,61	0,08	0,11	0,08	0,03
Cortisol	0,20	0,42	0,87	-0,55	-0,17	0,80	0,31	0,22	0,90
Prolactin	-0,02	-0,13	-0,12	0,26	-0,14	0,36	-0,02	-0,03	-0,19
Eigenvalues	0,63	0,34	0,22	0,46	0,34	0,17	0,89	0,28	0,20
Contribution to total variation (%)	46	25	17	43	32	16	62	19	14
Cumulative (%)	46	71	88	43	74	90	62	81	95

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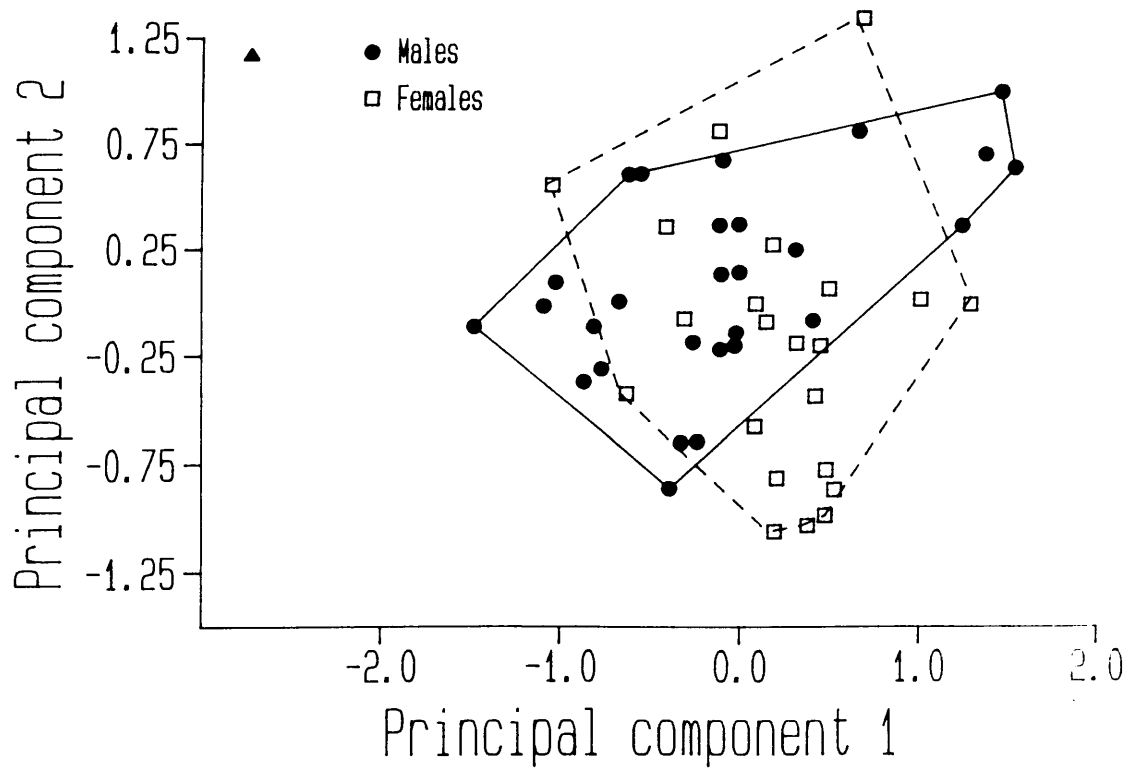
Fig. 22. Graphical representation of the variance around the first two PCs for the complete sample. The sexes are included in convex hulls (Jolliffe 1986). On outlier was identified (▲) and omitted from further analyses.

A graphic representation of the variance around the first two PCs of the complete sample is given in Fig. 22. Although there is a lot of overlap between the sexes it does appear that the two sexes show variances in different directions. The males show a larger lateral distribution (PC-1) whereas the females show a large vertical distribution (PC-2). This suggests that the sexes should probably be analysed separately.

The separate analysis of the sexes confirmed the trend observed in the above analysis, that the sexes were subject to different sources of variation, as their principal axes differed significantly (Table 4). In the females PC-1 consisted of a contrast between high androstenedione and low cortisol concentrations. The PC-2 showed a strong testosterone and oestradiol-17ß effect, whereas PC-3 had a large variance of cortisol, and

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some contribution from androstenedione and prolactin. In males, PC-1 showed a strong testosterone and androstenedione effect, PC-2 was the result of a strong contrast between testosterone and androstenedione, while PC-3 was a strong contrast between high cortisol and low androstenedione concentrations.
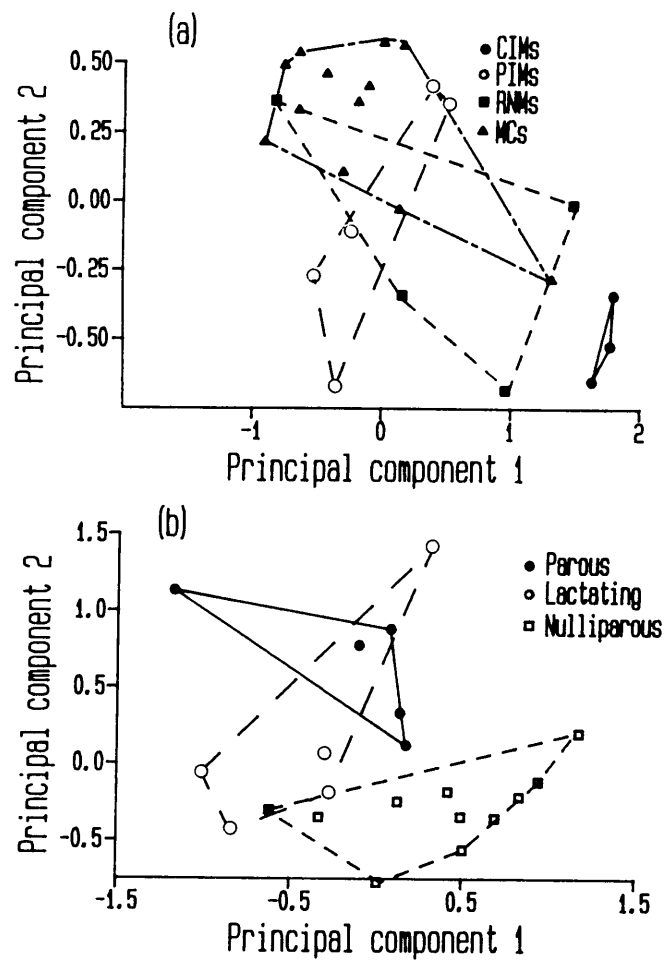
Fig. 23. Graphical representations of the variances along PC-1 and PC-2 for (a) males and (b) females. Those categories which provide the highest resolution in each sex are included in convex hulls (Jolliffe 1986).

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The visual display of the first two PCs also revealed some interesting trends. In the males a clear developmental axis emerged with most of the male cubs concentrated at the top of the spread, and the mating males (CIMs) near the bottom right. In between these two extremes there was a clear concentration of the RNMs with a tendency to cluster nearer the mating males. However, the PIMs showed very little variance along PC-1, but showed a larger variance along PC-2 (Fig. 23a).

The females on the other hand showed a clear separation between the NFs and the adult females. Both the NFs and the PFs showed large variances along PC-1, whereas the LFs showed their largest variance along PC-2.

Discussion

Although there were a large number of significant differences between means (Fig. 21) these were comprehensively discussed in the previous chapters. However, when using 'artificial' or imposed classification systems such as the reproductive and social categories used in this study, to compare trends in mean differences, there is a danger that a combined effect of both classification systems is in operation. A multivariate analysis using both classification systems to test for mean differences would therefore represent a repetitive analysis of the same effect. Were a combined effect present, the classification system would have to be re-evaluated or re-described. As no combined effect or interaction between the two classification systems was evident (see MANOVA), this possibility is removed. Also, the MANOVA provides an easy method for identifying important trends in the mean values of any multivariate problem. It is clear from the graphical representation of the MANOVA that the testosterone concentrations

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in CIMs, the oestradiol-17β levels in PFs, and the prolactin concentrations in LFs represent extreme mean endocrine conditions in this species, whereas mean cortisol concentrations do not differ significantly between the various categories investigated.

However, although these trends in mean values are indicative of mean hormonal changes associated with various social and reproductive categories, the nature of the variance within those categories is not elucidated. Principal component analysis was therefore performed in order to gain some insight into the nature of multidimensional endocrine variance during the socio-ecological development of spotted hyaenas.

The PC-1 calculated for males indicates that the total androgen concentration is the major hormonal variable identified in the present study for males. The importance of this variable is confirmed by PC-2, which is an indication of the males ability to secrete testosterone rather than androstenedione, and probably reflects the switch in the testosterone: androstendione ratio at puberty (chapter 3). When the androgen secreting abilty of the male has been accounted for (PC-1 & 2), the major source of variation within the male subsample is a contrast between high cortisol and low androstenedione concentrations. Whether this variable indicates a change in the steroid secreting ability of the adrenal remains to be investigated. The MCs and the RNMs displayed a large variance in both PC-1 and PC-2. However, the CIMs failed to show any significant variation along any of these axes. This could be an effect of sample size although there does seem to be a clear clustering of this category. The PIMs on the other hand showed little variance in terms of the total androgen secreting ability (PC-1), but did show a lot of variance in the ability to secrete

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either testosterone or androstenedione (PC-2). This suggests that although the potential ability to secrete androgens was present, very little androgen was in actual fact produced in these males subjected to prolonged social stress (Henschel & Skinner 1987). The variance observed in this social category was found to be the highest of all the adult males when PC-1 was plotted against PC-3, which suggests that there was a strong contrast between high cortisol and low androstenedione in this social category. Again this indicates a social inhibition of androgen secretion in these males, probably due to the effects of cortisol secretion (Sapolsky 1982).

The PC-1 for the female sub-sample which contrasts high androstenedione with low cortisol levels is similar to the PC-3 defined for males, however, the largest variance along this component is shown among the FCs and PFs. This suggests that there is an inverse relationship in terms of the ability to secrete these hormones, and again as was found for males, it may be that this in an indication of a change in steroid secreting ability, possibly in the adrenal. The LFs showed their largest variance along PC-2 which has a strong testosterone and oestradiol-17ß effect and a slight contrast with the stress-related hormones cortisol and prolactin. This latter observation is probably a function of the low prolactin levels found in this category (see MANOVA), but it also shows that a significant degree of ovarian activity occurs in this reproductive category, as there is a lot of variance in the amount of testosterone and oestradiol-17ß produced in this category (see chapter 4). At this stage it is difficult to interpret PC-3 in the females which comprises a strong cortisol effect and some positive contributions from the hormones androstenedione and prolactin.

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Thus, the MANOVA performed in this chapter identified mean testosterone concentrations in the CIMs , mean oestradiol-17β levels in PFs, and mean prolactin levels in LFs as extreme endocrine conditions for this species. However, in an investigation of multidimensional variance the ability to secrete large quantities of androgens was identified as the most significant variant describing male social development. Furthermore, the nature of hormonal variance suggests that the ability to secrete androgens is strongly related to circulating cortisol concentrations. The endocrine dynamics of female socio-ecological development appears to be quite different. The most significant trend was a strong contrast between high androstenedione and low cortisol concentrations, and although an adrenal origin for this pattern seems logical, this aspect still needs to be investigated.

In conclusion, the patterns identified in this multivariate investigation of endocrine variation during the social development of the spotted hyaena, clearly illustrates that the comparison of means (MANOVA) and an investigation of the nature of hormonal variation (PCA) identify different aspects of the total endocrine dynamics of the species. Therefore, a comprehensive analysis of both mean hormonal values and the nature of endocrine variance could make a considerable contribution towards our final understanding of the endocrine dynamics of this species.

CHAPTER 8 - EVOLUTIONARY CONSIDERATIONS

The spotted hyaena is unique among the Hyaenidae in terms of its ecology and a number of life history parameters. It is a social hunter and an opportunistic scavenger (Kruuk 1972, Henschel 1986), it usually produces precocial twins which are entirely dependent on milk obtained from the mother for up to one year of age (Kruuk 1972, Van Jaarsveld *et al.* 1988), resident females are dominant over immigrant males (Henschel & Skinner 1987), the external genitalia of females resemble those of males (Racey & Skinner 1979, Van Jaarsveld & Skinner 1987), and there is no clear sexual dimorphism in size in this species (Hamilton *et al.* 1986). This multitude of life history parameters makes for a complicated evolutionary scenario of this species, and this chapter contains some speculative arguments regarding a number of the issues mentioned above.

There is some evidence that access to food is a key resource for female spotted hyaenas, as their cubs are heavily dependent on milk (Kruuk 1972) and cub mortality can be affected by severe food shortages (Kruuk 1972, Knight *et al.* submitted). Females are also the more territorial of the two sexes (Henschel & Skinner 1987), and philopatric females form coalitions along matrilinear lines, for foraging purposes (Mills 1985), with the group size being related to prey size (Kruuk 1972). Furthermore, whole clans consist of closely related females that actively defend vital resources (Frank 1986a, Mills 1985), and clan fissions are usually along matrilinear lines (Mills 1985). On the other hand, males are generally denied access to carcasses, and consequently tend to scavenge more than females. This means that they seldom associate with groups and forage on their own. Also, they frequently transverse territorial boundaries in order to gain mating

opportunities, even at great risk to their lives (Frank 1986b, Henschel & Skinner 1987). This emphasises the importance of females as a key resource for males. This preliminary evidence indicates that there are different key resources for the two sexes and that the behavioural ecology, including investigations aimed at elucidating the origins of group hunting in this species, should be interpreted in a sex-specific fashion (Wrangham 1980).

One of the most interesting features of spotted hyaena society is that females are dominant over males (Kruuk 1972, Frank 1986b, Henschel & Skinner 1987). Female dominance was originally attributed to high levels of androgens in adult females resulting in a larger body size (Racey & Skinner 1979). However, this view was challenged on two fronts. First, a study of body morphometrics in this species (Hamilton *et al.* 1986) failed to show any difference in frame size between the sexes. This showed that although females were heavier than males they were not necessarily larger boned. This suggested that the larger body mass of females is the result, rather than the cause of dominance (Van Jaarsveld & Skinner 1987), and that it results from their preferential access to food (Hamilton *et al.* 1986). Second, endocrine studies have illustrated that adult females do not have high levels of circulating androgens (Van Jaarsveld & Skinner 1987, present study), but rather that the majority of adult males show a low androgen status (see chapter 7). If, as suggested above, a higher adult female than male mass is a consequence of dominance, female dominance in this species still needs to be explained. The high androstenedione concentrations observed in juvenile females (Glickman *et al.* 1987, present study) could result in a faster growth rate in female than male cubs due to androgenic effects (MacGillivary 1987). However, although females in mixed litters tend to grow faster and are dominant over males from an early age (personal

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observation), insufficient data exists to separate cause from effect. An intensive study which monitors dyadic dominance within mixed litters, milk consumption, plasma androstenedione concentrations and relative growth is required to untangle this problem.

The source of androstenedione in juvenile females also needs to be identified. There are currently two possibilities, either a gonadal or an adrenal origin. Increased androgen secretion from the prepubertal andrenal (Adrenarche), specifically from the zona reticularis, is a well documented phenomenon (MacGillivary 1987) and is thought to influence the timing of puberty. On the other hand, it is also known that pituitary FSH and LH concentrations rise soon after birth in humans (*Homo sapiens*), together with a testosterone response from the testis. This steroid response in males then causes a fast decline in pituitary activity due to its negative feedback effect. The ovary usually shows no endocrine response and as a result pituitary activity in females does not decline until the pre-pubertal nadir (Winter 1987). If the elevated androstenedione concentrations in juvenile females were of gonadal origin, the reasons for a lack of pituitary inhibition through negative feedback would have to be explained. This, together with the inverse relationship between androstenedione and cortisol secretion identified in the present study (chapters 5 & 7) suggests that an adrenal origin of androstenedione in juvenile females is a more likely alternative.

If the lack of sexual dimorphism in the spotted hyaena cannot be attributed to high adolescent or adult androgen concentrations in females, one other explanation for this phenomenon remains. That is, that there is no distinct sexual dimorphism because the anabolic androgenic effects at adolescence, that are usually responsible for the excessive male growth spurt in most

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male mammals, are absent in the spotted hyaena (Santen 1987). The present study provides some evidence in support of the latter hypothesis. First, only those males that have procured mating rites show elevated testosterone concentrations (chapters 3 & 7), while the other adult males show testosterone levels similar to those found in females. Second, changes in the concentrations of the stress-related hormones, cortisol and prolactin, follow patterns predicted by stressors associated with dispersal (chapters 5 & 6). Furthermore, this alternative hypothesis raises questions about the growth promoting effects of raised androstenedione concentrations in juvenile females. Is the growth promoting effect of the weak androgen, androstenedione, enough to promote growth in juvenile females, while the combined effects of lower androstenedione and higher testosterone (a strong androgen) concentrations in adolescent males fails to show any significant effect ? Therefore, present data suggest that a behavioural modificaton (i.e. increased aggression) in juvenile females due to high circulating androstenedione concentrations (Glickman *et al.* 1987), during a phase when extensive brain development occurs (Sapolsky & Meaney 1986) offers the better explanation of female dominance.

Although the origin of sexual monomorphism in the spotted hyaena has received a lot of attention (for review see Van Jaarsveld & Skinner 1987), possible explanations for the relative precociality of young (Van Jaarsveld *et al.* 1988) have not been put forward. Although several hypotheses for the altricial-precocial gradient in mammals have been proposed, including maternal body size, maternal metabolic rate (Martin 1981, Hofman 1983) and gestation length, i.e. high prenatal investment (Pagel & Harvey 1988), a recent investigation illustrated that relative gestation length in mammals shows a strong correlation with the relative rate of mortality. Moreover,

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relative mortality correlated with relative neonatal brain size, the latter character which is generally considered to be indicative of the altricial-precocial gradient in mammals (Pagel & Harvey 1990). These findings suggest that altricial species evolved shorter gestation periods and smaller-brained neonates, apparently in response to high rates of mortality, and other species evolved longer gestation lengths with larger-brained neonates in response to lower rates of mortality (Pagel & Harvey 1990). In view of the above hypotheses, Pagel & Harvey (1990) suggested that the advantages associated with faster reproduction in the face of increased mortality were obvious. However, the question remains to be answered: why does slower reproduction result in neonates with larger brains for their body size ? In the absence of evidence, two alternative explanations for the latter problem were suggested, namely, that larger brains at birth are a nonadaptive allometric consequence of a relative faster brain growth compared to the growth of the rest of the body during gestation in all mammals. This means that a longer gestation length will inevitably result in a relatively larger brain for any particular body size. The second explanation views increased neonatal brain size as an adaptive response to essentially the same forces that lead to increased gestation length, namely, that some species increase gestation length to produce fewer and larger offspring at an advanced state of development in response to excessive juvenile mortality rates (Horn 1978).

The first hypothesis could easily be tested provided sufficient data were available, while the second hypothesis, originally proposed by Horn (1978), is inherently difficult to test as measured population mortalities would reflect *post fact* mortality rates, or those after natural selection had eliminated excessive mortality in any particular life phase. However, the

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response of a species to a variety of, or any one unusual ecological condition may often be an indication of the nature of the selection pressures operating on population parameters. Although direct measures of spotted hyaena juvenile mortality under different ecological conditions are scarce, a few studies have indicated that juveniles may be very vulnerable. Kruuk (1972) observed that juvenile mortality was higher in the Serengeti than in the Ngorongoro crater. In Ngorongoro, the clans are sedentary, while females in the Serengeti have to travel large distances to follow migrating game populations, and consequently often stay away from their dens up to five days (Kruuk 1972, East *et al.* 1989). Similarly, a clan studied in the Kruger National Park (Henschel 1986) failed to raise a single litter from a minimum of eight detected pregnancies, and only one litter survived for longer than two weeks. This failure to raise litters, mainly due to lion predation, resulted in the eventual demise of the clan. Lion predation is also seen as the principle factor responsible for cub mortalities in East Africa (East *et al.* 1989).

In another study (Knight *et al.* submitted), a marked increase in cub mortality in the Kalahari Gemsbok National Park coincided with an environmental perturbation, and an interesting behavioural observation. In a clan where the average cub mortality was 10,5 % during the years preceding a major drought (1978-1984), it increased dramatically to an average mortality of 46,5 % during the drought period (1985-1986). During this period allo-suckling was observed for the first time in a species well documented for the fact that mothers suckle only their own young (Kruuk 1972, Mills 1985, East *et al.* 1989). This interesting example of behavioural flexibility in the spotted hyaena coinciding with high cub mortalities supports the above data in suggesting that this precocial

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carnivore is particularly vulnerable to excessive juvenile mortality. Therefore, the hypothesis that high juvenile mortalities result in a selection pressure for precocial young in mammals (Horn 1978) could very well hold for the sole precocial fissiped carnivore, the spotted hyaena.

Another factor that may significantly affect juvenile mortality in this species is the suggested incidence of siblicide (Frank 1989, Hofer & East 1989). Although this would generally affect juvenile mortality, it is important to note that most of the evidence for siblicide is circumstantial. Moreover, there is a possibility that these cub mortalities in the wild are actually inflicted by other cubs or males, not necessarily siblings. This latter view also supports one of the hypotheses originally proposed by East *et al.* (1989), namely that the function of birth dens in this species is to protect young from other cubs, until they are old enough to defend themselves. In this regard, it is significant that birth dens are not common in areas where smaller clan sizes do not result in large communal dens (Mills 1990).

SUMMARY

The aims of the present study were to investigate the patterns of endocrine secretion during the socio-ecological development of *Crocuta crocuta*. Patterns of testosterone secretion revealed a larger variance in circulating testosterone within the sexes than between them. The observed differences between males was largely determined by the pattern of socio-ecological development of the species, whereas differences between females seemed to be related to changes in ovarian activity. Moreover, the very high testosterone and androstenedione concentrations found in those males that have been accepted into target clans after dispersal, explains why most studies to date have failed to demonstrate any significant differences in peripheral androgen concentrations between the sexes. Also, the biological availability of circulating testosterone to adult females may be significantly lower than would be deduced from an analysis of organically extracted plasma, due to significant plasma testosterone binding. Patterns of androstenedione secretion differed markedly from those found for testosterone. Although androstenedione concentrations followed observed testosterone patterns in males, secretion of these two hormones were not correlated in females. Plasma androstenedione concentrations were the highest in juvenile females, which suggests that elevated androstenedione levels may play a role in the establishment of behavioural dominance of female over male spotted hyaenas.

It was not possible to distinguish between females belonging to either the parous, nulliparous or lactating reproductive categories on the basis of circulating progesterone concentrations. Similarly, although there was a trend towards reduced oestradiol-17ß concentrations during lactation, this

did not reach significant levels. Consequently both the patterns of progesterone and oestradiol-17ß secretion confirm the absence of any distinct lactational anoestrus in this species. A strong positive correlation observed between circulating oestradiol-17ß concentrations and plasma testosterone binding ability in adult females confirm earlier suggestions that female dominance in the spotted hyaena is not the result of high concentrations of circulating androgens in adult females (Van Jaarsveld & Skinner (1987).

Adreno-cortical responsiveness in this social carnivore supports some of the rodent and primate literature, in that no distinct difference could be detected in those animals experiencing social turmoil. In addition, the trend towards reduced basal cortisol concentrations in male and female cubs was not significant, suggesting that the reduced cortisol levels during postnatal stages in the rat (Sapolsky & Meaney 1986), is not as pronounced in this carnivore. Also, the pattern of prolactin secretion failed to show any significant change during male dispersal, or among vatious dominance related social categories. Moreover, the negative relationship recorded between prolactin and cortisol in this social carnivore does not support the hypothesis that prolactin acts as a protective agent against glucocorticoid action (Drago *et al.* 1989). In female spotted hyaenas the lactating females exhibited the lowest prolactin concentrations. This suggests a prolactin pattern in this species, similar to that previously observed in rabbits and brown hares (McNeilly & Friesen 1978, Mondain-Monval & McNeilly 1990), which may be the result of infrequent suckling patterns. Moreover, this also explains the lack of any distinct lactational anoestrus in this polyoestrous aseasonal breeder.

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MANOVA identified the high testosterone concentrations found in **CIMs**, the high oestradiol-17β concentrations in PFs, and the low prolactin concentrations in LFs as extreme mean endocrine conditions for this species. However an analysis of the nature of endocrine variance indicated that the ability to secrete androgens and the contrast between androstenedione and cortisol were the principal axes in describing endocrine variance for male and female spotted hyaenas respectively. Therefore, a comparison of means and an analysis of the nature of endocrine variance can be complementary in unravelling the endocrine dynamics of a species.

OPSOMMING

Die doel van die huidige studie was om die patroon van hormonale afskeiding tydens die sosio-ekologiese ontwikkeling van *Crocuta crocuta* te ondersoek. Testosteroon afskeiding toon aan dat daar 'n groter variasie binne die geslagte as tussen hulle bestaan. Die waargenome verskil tussen mannetjies was hoofsaaklik deur die sosio-ekologiese ontwikkelingspatroon van die spesie bepaal, terwyl die variasie wat by wyfies opgemerk is hoofsaaklik te wyte was aan sikliese ovarium aktiwiteit. ie hoë testosteroon en androsteendioon konsentrasies in daardie mannetjies wat reeds in 'n teiken familietrop aanvaar is, verklaar waarom die meeste vorige studies nie 'n verskil in androgeenvlakke tussen tussen die geslagte kon waarneem nie. Verder is die biologiese beskikbaarheid van sirkulêrende testosteroon vir volwasse wyfies heel moontlik laer as wat mens sou verwag na aanleiding van die analiese van organies ge-ekstraeerde plasma, as gevolg van die betekenisvolle plasma binding van die hormoon. Alhoewel androsteendioon vlakke die van testosteroon konsentrasies navolg in die mannetjies was die twee hormone nie gekorreleeer in die wyfies nie. Plasma androsteendioon konsentrasies was die hoogste in jong wyfies, wat daarop dui dat die hoër 'n rol tydens die daarstelling van die dominansie van wyfies oor mannetjies kan speel.

Dit was nie moontlik om tussen wyfies wat tot die gepaarde, ongepaarde en lakterende voortplantings kategorïee behoort, te onderskei deur middel van sirkulêrende progesteroon konsentrasies nie. Verder was daar 'n neiging tot verlaagde estradiol-17ß konsentrasies gedurende laktasie wat nie betekenisvolle vlakke bereik het nie. Dus bevestig die patroon van beide progesteroon en estradiol-17ß afskeiding die afwesigheid van 'n duidelike

ovariale inhibisie tydens laktasie. 'n Sterk positiewe korrelasie tussen die sirkulêrende estradiol-17β konsentrasies en plasma testosteroon binding in volwasse wyfies bevestig verder vroëer voorstelle dat die dominansie van wyfies oor mannetjies nie die gevolg van hoë sirkulêrende androgeen vlakke in die volwasse wyfie is nie (Van Jaarsveld & Skinner 1987).

Die vermoë van die gevlekte hïena se adrenaalkorteks om kortisol af te skei bevestig sommige van die primaat literatuur deur dat geen betekenisvolle verskil in daardie diere wat sosiale skommelings ondervind waargeneem is nie. Die neiging na verlaagde basale kortisol vlakke in afhanklike mannetjie en wyfie kleintjies was nie betekenisvol nie en dui daarop dat die baie lae kortisol vlakke waargeneem tydens post-natale ontwikkeling in die rot (Sapolsky & Meaney 1986) nie so beduidend in diè karnivoor is nie. Verder het daar geen betekenisvolle verandering tydend manlike verstrooïng, of tussen verskeie dominansie verwante sosiale kategorieë voorgekom nie. Die negatiewe verwantskap tussen prolaktien en kortisol ondersteun ook nie die hipotese dat prolaktien die effekte van kortisool afskeiding teenwerk nie. In wyfie gevlekte hïenas het die lakterende wyfies die laagste prolaktien vlakke vertoon. Dit impliseer dat 'n prolaktien patroon wat soortgelyk is aan die wat voorheen in konyne en hase beskryf is (McNeilly & Friesen 1978, Mondain-Monval & McNeilly 1990) en die gevolg van 'n onreelmatige soogpatroon is, by die gevlekte hïena aanwesig is. Dit verklaar ook die afwesigheid van enige definitiewe ovariale inhibisie tydens laktasie in hierdie spesie.

MANOVA het die hoë testosteroon konsentrasies in sentrale immigrerende mannetjies, die hoë estradiol-17β vlakke in gepaarde wyfies, en die lae prolaktien konsentrasies in lakterende wyfies as ekstreme hormonale

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kondisies in die spesie ge-identifiseer. 'n Analiese van die aard van hormonale variasie het die vermoë om androgene te kan produseer en die kontras tussen androsteendioon en kortisol produksie as die vernaamste asse wat hormonale variasie in manlike en vroulike gevlekte hïenas respektiewelik beskryf, ge-identifiseer. Dus kan beide die vergelyking van gemiddelde waardes en 'n analiese van die aard van hormonale variasie bydrae tot die blootlegging van die dinamika van hormonale prosesse in 'n spesie.

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Immobilization and anaesthesia of spotted hyaenas, *Crocuta crocuta*

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A single intra-muscular dose of 3 mg etorphine hydrochloride (M99) and 30 mg xylazine hydrochloride was found effective for the immobilization of wild free-living spotted hyaenas *Crocuta crocuta* (47–72 kg). Animals (*n* = 5) went down within 2 min 44 s ± 51 s and because of the possibility of using an M99 antagonist, the drug combination used is relatively safe. Anaesthesia could be maintained for extended periods (4 h) using halothane.

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'n Enkele binnespierse dosis van 3 mg etorfienhidrochloried (M99) en 30 mg xylazienhidrochloried was effektief vir die immobilisasie van wilde vrylewende gevlekte hiënas *Crocuta crocuta* (47–72 kg). Die diere (*n* = 5) het binne 2 min 44 s ± 51 s gaan lê, en as gevolg van die moontlikheid om 'n M99-antagonis te kan gebruik, is dié kombinasie relatief veilig. Narkose kon vir lang periodes (4 h) met halotaan gehandhaaf word.

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Introduction

Spotted hyaenas, *Crocuta crocuta*, have been immobilized with succinylcholine chloride, phencyclidine hydrochloride and ketamine hydrochloride (Kruuk 1972; Ebedes 1973; Smuts 1973; Harthoorn 1976). The disadvantages associated with the use of succinylcholine chloride and phencyclidine hydrochloride have been reported on by Smuts (1973) and Lindeque (1981).

This investigation was carried out for proposed future endocrinological studies on spotted hyaenas. In order to achieve serial blood sampling in this species, an immobilization procedure had to be established which incorporates a short induction time, and is followed by prolonged anaesthesia. The present paper reports on the establishment of an effective field immobilization procedure following the investigation of several drug combinations during 1983 and 1984.

Materials and Methods

Ketamine hydrochloride

Four spotted hyaenas were immobilized with an intramuscular injection of ketamine hydrochloride (Ketalar, Parke Davis Laboratories (Pty) Ltd, Isando) and xylazine hydrochloride (Rompun, Bayer Pharmaceuticals (SA) (Pty) Ltd, Johannesburg) at the Johannesburg Zoological Gardens (*n* = 2) and Kruger National Park (*n* = 2), using Cap-Chur immobilizing equipment (Palmer Chemical & Equipment Co., Inc., Douglasville, Georgia, USA). Following immobilization, anaesthesia was maintained in the free-living animals for 40 min and 1 h 46 min respectively by the intra-venous administration of 100–500 mg of ketamine hydrochloride when deemed necessary. In the two captive animals, anaesthesia was maintained for 2 h 56 min with a combiation of 9% alphaxolone and 3% alphadolone (Saffan, Glaxo-Allenburys (SA) (Pty) Ltd., Germiston) and for 6 h with halothane (Fluothane, ICI Pharmaceuticals Ltd., Johannesburg) using a circle absorber machine (Fluotec, Cyprane Ltd, Keighley, England) respectively.

Etorphine hydrochloride (M99) and halothane

Sixteen free-living spotted hyaenas were immobilized with 3 mg M99 (Rickett & Coleman Pharmaceuticals Div., Pinelands) in the Kruger National Park using Telinject immobilizing equipmment (Telinject S.A., Randburg). In 14 animals the darts all included 30 mg xylazine hydrochloride; the remaining were given 35 mg azaperone (Azaperone Vet., Ethnor Laboratories (Pty) Ltd, Johannesburg).Where required, an additional 30 mg xylazine hydrochloride was administered after capture (*n* = 3). Following immobilization 11

animals were intubated with an 11 mm diameter endotracheal tube and anaesthesia was maintained with halothane using a circle absorber machine. Induction was achieved with a 10% mixture of halothane and oxygen and the animals were maintained using 2–4% halothane in a closed circuit. The antidote was administered gradually 23 min – 1 h 48 min after immobilization (1 h ± 25 min; $n = 11$) while monitoring the respiration rate and adjusting the halothane concentration to maintain anaesthesia. After these animals had reached a stable plane of anaesthesia the M99 antagonist, diprenorphine (M5050, Rickett & Coleman) was administered intravenously. Respiration rate, pulse rate and rectal temperatures were randomly monitored during prolonged anaesthesia. Respiration was stimulated using doxapram hydrochloride (Dopram, A.H. Robins, Div., Keatings Pharmaceuticals Ltd, Johannesburg) when required ($n = 2$) and body temperature was maintained using hot water bottles and/or solar blankets.

All immobilized animals were injected (i.m.) with 4 ml of a long-acting penicillin (Lentrax, May & Baker Ltd, Dagenham, England) and superficial wounds were treated with an antibiotic (Furacin ointment, Smith, Kline and French (Pty) Ltd, Isando).

Results and Discussion

Ketamine hydrochloride

The administration of ketamine hydrochloride results in a smooth immobilization with a relatively long induction time (Table 1), which increases the risk of losing sight of the animal following darting. The large volume required for successful immobilization results in heavy darts which also increases the post-darting flight reaction. The quantities of ketamine hydrochloride required for prolonged anaesthesia (Table 1) were considered too costly. Anaesthesia with halothane after immobilization was found to be impractical in the field, owing to the persistance of the laryngeal and pharyngeal reflexes after ketamine hydrochloride immobilization (Gillmann, Goodman & Gillman 1980).

Saffan

The single animal maintained with saffan (Table 1) showed

respiratory depression and cyanosis. Single doses larger than 2 ml (i.v.) resulted in hypopnoea. There were no clinical indications of an allergic reaction to this drug.

Etorphine hydrochloride (M99) and halothane

Etorphine hydrochloride immobilization at a dosage rate of 3 mg per animal resulted in a short induction time (Table 2), characterized by an excitatory period lasting approximately 20 s, during which the animal moved from 20–150 m. In a single case, the reduction of the darting dose to 2 mg increased the excitatory phase to approximately 6 min. A dose of 4 mg resulted in apnoea, which was rectified using artificial respiration and the intravenous administration of 4 ml doxapram hydrochloride ($n = 2$). A single immobilization with M99 only, resulted in the absence of palpebral and laryngeal reflexes. The animals were insensitive to light and sound and they usually defaecated during immobilization. Green (1976) found that dingos, *Canis familiaris dingo*, immobilized with M99 and acetylpromazine, defaecated frequently and were sensitive to sound.

With the M99 and azaperone combination or with lower doses of xylazine hydrochloride together with M99, spotted hyaenas were extremely sensitive to touch and also displayed paddling motions of the legs. This was prevented by using 30 mg of xylazine hydrochloride with the etorphine. In a few instances the hyaenas developed clinical signs of cyanosis as a result of falling with their noses into the ground, which was easily rectified by changing their postures but which could possibly have resulted in one suspected casualty when an animal could not be found following immobilization. In another instance an overdose was administered owing to non-intramuscular darting which resulted in cardiac failure. Antidote administration following M99 and xylazine hydrochloride capture resulted in full recovery within 33 s – 4 min 30 s (2 min 32 s ± 1 min 51 s; $n = 5$).

The absence of the laryngeal reflex made intubation easy. Owing to the absence of the palpebral and interdigital reflexes, a combination of tissue colour and respiration rate was used to ascertain the depth of anaesthesia. The administration of the antidote caused a sharp increase in respiration rate, which

Table 1 Anaesthesia of four spotted hyaenas using ketamine hydrochloride, xylazine hydrochloride and Saffan (SD = standard deviation; n = sample size; min: s = time expired after darting)

		Darting dose (mg/kg)					Maintenance dose (mg/kg/h)	
	Mass (kg)	Ketamine hydrochloride	Xylazine hydrochloride	Ataxia (min:s)	Recumbance (min:s)	Tractable (min:s)	Ketamine hydrochloride	Saffan
n	4	4	4	4	4	4	17	7
Mean ± SD	62,3 ± 6,7	10,7 ± 1,9	0,5 ± 0,1	2:30 ± 0:36	7:00 ± 2:36	10:30 ± 4:06	20,6 ± 11,7	2,17 ± 2,5
Range	53,5 – 69,5	9,5 – 12,3	0,5 – 0,6	2:00 – 3:00	4:00 – 10:00	6:00 – 14:00	10,8 – 51,6	0,4 – 7,8

Table 2 Immobilization of 16 spotted hyaenas using etorphine hydrochloride (M99) together with xylazine hydrochloride or azaperone (SD = standard deviation; n = sample size; min:s = time expired after darting)

		Dose (mg/kg)					
	Mass (kg)	M99	Xylazine hydrochloride	Azaperone	Ataxia (min:s)	Recumbance (min:s)	Tractable (min:s)
n	16	16	13	3	7	5	5
Mean ± SD	60,39 ± 6,76	0,05 ± 0,01	0,63 ± 0,23	0,57 ± 0,02	1:30 ± 0:42	2:17 ± 0:26	2:44 ± 0:51
Range	47,0 – 72,0	0,04 – 0,06	0,32 – 0,99	0,55 – 0,56	0:32 – 2:35	2:00 – 3:26	2:00 – 4:00

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illustrates the depression of the respiratory centres caused by M99 (Harthoorn 1976). The animals were taken off halothane after 4 h and were ambulant after 40 min − 2 h 1 min (1 h 5 min ± 29 min; $n = 8$). Some individuals ($n = 3$) fell asleep during the recovery period.

Conclusion

Three milligrams of M99 with 30 mg xylazine hydrochloride is a suitable combination for the effective immobilization of wild free-living spotted hyaenas, and the presence of an antagonist (M5050) makes it ideal for short-term capture or for initiation of prolonged anaesthesia with halothane.

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Bone-collecting habits of spotted hyaenas *Crocuta crocuta* in the Kruger National Park

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An examination of 18 *Crocuta crocuta* dens in the Kruger National Park, showed that this species can be responsible for bone assemblages at dens, more so at permanent sites such as granite or calcrete caves than at temporarily occupied aardvark holes or road culverts. Because of the facultative nature of food-transporting behaviour, the rate at which bones are accumulated at dens varies, ranging from no bones collected to nine food items collected per month. The relative abundance of ungulate species represented in the large assemblages resembles that of ungulates living in the region surrounding a den.
S. Afr. J. Zool. 1986. 21: 303 – 308

Agtien *Crocuta crocuta* lêplekke is in die Nasionale Krugerwildtuin ondersoek. Uit die resultate blyk dat hierdie spesie soms bene in sulke gate versamel, veral in permanente graniet- of kalkreetgrotte en minder dikwels in tydelike erdvarkgate of stormwatersiote. Die tempo waarteen hierdie beenversamelings groei varieer van geen tot nege items per maand, hoofsaaklik te wyte aan die onvoorspelbare wyse waarop hiënas die oorskotte van prooidiere versprei. Die relatiewe voorkoms van prooispesies in die beenversamelings vergelyk goed met die voorkoms van prooidiere in die onmiddellike omgewings van die lêplekke.
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Whether or not spotted hyaenas *Crocuta crocuta* accumulate bones at den sites has long been a subject of dispute (Hughes 1954; Brain 1981). Originally, part of this controversy arose because of interspecific behavioural differences between hyaenids. It has now been established beyond doubt that both *Hyaena hyaena* and *H. brunnea* are important bone-collecting agents (Kruuk 1976; Skinner 1976; Mills & Mills 1977; Skinner, Davis & Ilani 1980; Skinner & van Aarde in press) and that the porcupine *Hystrix africaeaustralis* is an important secondary agent which frequently serves to confound the analysis of bone assemblages (Brain 1976; Maguire 1976).

In the present paper, we contribute to the ongoing discussion on the role of spotted hyaenas as agents responsible for bone accumulations (see Mills & Mills 1977; Henschel, Tilson & von Blottnitz 1979; Brain 1981) by presenting results of an analysis of bone assemblages at 18 dens in the Kruger National Park. Because brown hyaenas are extremely rare in this park (Mills 1985), this analysis presents a good opportunity to analyse the bone-collecting habits of spotted hyaenas with little chance of confusing them with those of brown hyaenas.

Apart from documenting the nature of the assemblages and the type of cavity used by spotted hyaenas, factors which can be useful for determining the probable collecting agent at palaeontological sites (Brain 1981), this study presents notes on the rate of bone accumulation by spotted hyaenas. Furthermore, the hypothesis that ancient ungulate fauna can be reconstructed from an analysis of bones found in ancient hyaena dens is tested by comparing the species compositions of bones found at three of the present study sites with those of living ungulates in the region.

Methods

Eighteen spotted hyaena dens were examined along the length of the park (Table 1). Whenever possible, bones were examined and identified on site, but others were removed for later analysis. At one den, observations were conducted on the rate of bone accumulation over a one-month period. Other observations of hyaenas of one clan transporting animal remains, were made over a two-year period.

Data on the abundance of ungulates of impala (*Aepyceros melampus*) size and bigger during six consecutive years (1979 to 1984) in the vicinities (31,5 – 50,4 km²) of three den sites were obtained from the National Parks Board data bank of aerial censuses conducted once annually between May and July.

Table 1 Location of spotted hyaena dens examined

Den No. Location	Longitude	Latitude
1 Olifants Rest Camp	31 46 E	24 46 S
2 Nwatimhisi (Olifants)	31 40 E	24 00 S
3 Mbangari (Roodewal)	31 37 E	24 12 S
4 Nhlanganini (Letaba)	31 33 E	23 57 S
5 Josiaspruit (Pafuri)	31 15 E	22 28 S
6 Mananga (Satara)	31 48 E	24 20 S
7 Witpens (Satara)	31 44 E	24 21 S
8 Olifants Trails Camp	31 45 E	24 00 S
9 Kingfisherspruit	31 27 E	24 27 S
10 Orpen	31 26 E	24 29 S
11 Punda Milia	30 55 E	22 43 S
12 Manangananga	31 03 E	22 49 S
13 Olifants River Bridge	31 42 E	24 04 S
14 Tshokwane	31 52 E	24 48 S
15 Tshokwane	31 44 E	24 42 S
16 Pretoriuskop	31 16 E	25 09 S
17 Napi Plots	31 24 E	25 06 S
18 Nghotsa	31 43 E	24 13 S

Results

Den descriptions

Four types of dens were used by spotted hyaenas in the Kruger National Park. These were granite caves formed by weathering, eroded calcrete limestone cavities, earthen holes probably excavated by aardvark (*Orycteropus afer*), and concrete culverts under macadamized roads (Table 2; Figure 1; Figure

2). All 18 hyaena dens examined had more than one entrance. The sizes of all dens were such that adult hyaenas could enter for some distance, but all natural caves, except one granite and two earthen dens, had smaller side tunnels which could only be entered by cubs. The entrances of granite and calcrete caves were high (0,6 – 1,4 m), but the caves usually narrowed down to less than 0,2 m. Earthen dens were sometimes intricate systems, with numerous entrances and a network of tunnels, the height of the main tunnel being 0,3 – 0,5 m.

Three of the four granite caves examined showed signs of porcupine occupation, judging by the number of quills and porcupine faeces found inside. Except for den No. 6, where an aardvark resided at one end of the system, while hyaenas made use of three other entrances, no evidence of co-habitation was found at any of the earthen dens. Culverts were also used by warthogs, porcupines, bats and jackals, but no evidence of co-habitation with spotted hyaenas was found.

Bone assemblages

The number of bones found at different dens varied substantially (0 – 626; $\bar{x} = 48,67 \pm 145,73$; Table 2). Granite and calcrete caves usually had large assemblages ($\bar{x} = 158,40 \pm 262,67$), while earthen dens and culverts seldom had more than an occasional bone ($\bar{x} = 6,46 \pm 11,89$). Six dens (1, 2, 3, 5, 6 & 13) had more than 28 bones, but others had less than nine.

The results of the three biggest collections at Dens 1, 3 and 5 are summarized in Table 3. Although all three dens were also inhabited by porcupines, only 1,3%, 13,6% and 15,0%
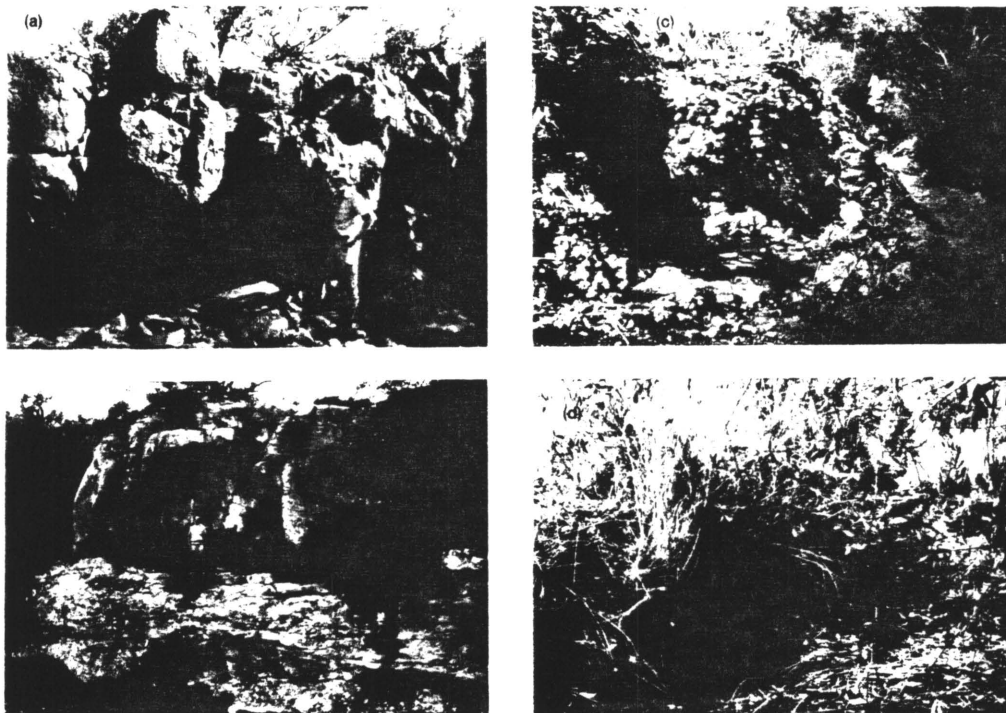

Figure 1 Entrances of natural hyaena den sites utilized by spotted hyaenas in the Kruger National Park at: (a) Olifants Site 1; (b) Mbangari Site 3; (c) Pafuri Site 5; (d) Satara Site 6.

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Table 2 Descriptions of spotted hyaena dens examined in 1983 and 1984. Source of information for last occupation by spotted hyaenas are: o = own observation; r = ranger report; f = footprint; s = hyaena scat; h = hyaena hair identified by cross-sectioning (Keogh 1979); maximum tunnel height was measured at a position 2 m into a den

| No.	Substratum	Origin	Number of Entrances	Tunnel Height	Bones	Last occupied	Source
1	Granite	Wind?	2	80 cm	626	pre-1984	r,s
2	"	Water	2	100 cm	41	pre-1984	r,s
3	"	"	2	60 cm	44	pre-1984	r,s,f
4	"	"	2	35 cm	4	1983	o
5	Calcrete	"	2	24 cm	77	pre-1983	r,f,h
6	Earth	Aardvark	3	30 cm	36	1984	o
7	"	"	2	50 cm	2	1983	o
8	"	"	8	30 cm	0	1984	o
9	"	"	4		8	1982	r
10	"	"	2		0	1981	r
11	"	"	2		0	1984	r,f
12	"	"	4		1	1983	r
13	Culvert	Man	2		29	1982	o
14	"	"	2		0	1983	o
15	"	"	2		0	pre-1984	r
16	"	"	2		5	pre-1984	r
17	"	"	4		0	1984	o
18	"	"	2		3	1984	o
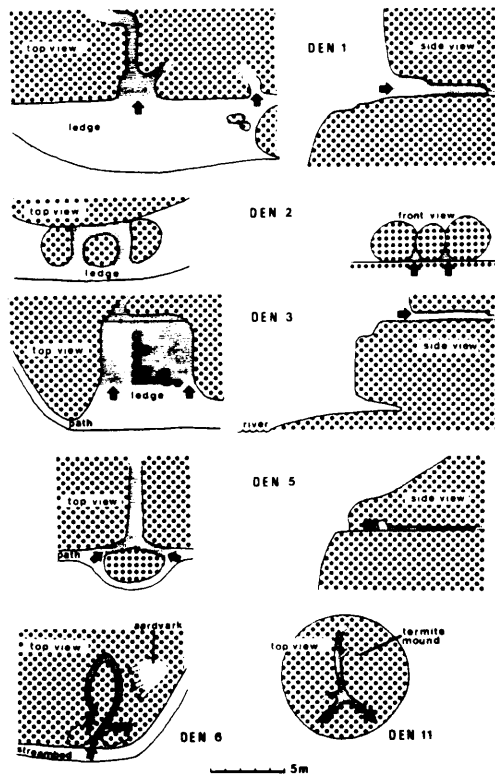
Figure 2 Schematic drawings of horizontal sections (top view), vertical sections (side view), or front view of six natural spotted hyaena dens (shaded). Arrows indicate den entrances.

respectively of the bones were gnawed by porcupines, while all showed signs of crushing or gnawing by hyaenas. These assemblages consisted mostly of long bones (Table 3; Appendix). Of the 41 bones found at Den 2, 39 were unidentified chips and two were impala leg bones. The 29 bones at Den 13 comprised 6 of impala, 3 of zebra, 1 of giraffe and 19 unidentified chips.

The relative abundance of the major prey species in the assemblages at Dens 1, 3 and 13 was compared to the relative mean abundance of ungulates in the areas surrounding these dens (Figure 3). The values of Spearman Rank correlation coefficients were highly significant ($P < 0,01$; r_s = 0,84, 0,77 and 0,89 for Den 1, 3 and 13 respectively), although by visual analysis, giraffe seemed to be more abundant in the assemblage at Den 3 than would be expected from the surrounding fauna. This was probably related to the large size of the giraffe bones, which enhanced their survival rate. These results indicate that the bone assemblages gave a fairly accurate representation of the surrounding medium-to-large ungulate populations.

Rate of accumulation

The rate of bone accumulation was monitored at an earthen den (No. 6) which hyaenas had adapted from an actively used aardvark den. During a month-long period of observation at this den, where a female hyaena had given birth to a cub, the dam carried ungulate remains to the den on eight occasions, and her adult male companion did so once. All nine items were complete fleshy legs, scapula (or humerus) to hoof or femur to hoof, and in one case the intact hindquarters of a zebra foal was taken to the den (Table 4). In three instances these items were carried from carcasses which were more than 5 km from the den. The dam fed on all of these items at the den, while other hyaenas occasionally partook of them. When the dam died violently after a month, examination of the den revealed the dismembered remains of seven of these items inside or within 10 m of the den (Table 4).

Table 3 Species distribution of bones inside (i) and within a 10 m radius outside (o) of three dens in the Kruger National Park; totals (t) and percentages (%) are indicated

Species	Den sites 1				3				5			
	i	o	t	%	i	o	t	%	i	o	t	%
Giraffa camelopardalis	15	3	18	2,9	8	5	13	29,6				
Syncerus caffer	17	2	19	3,0	6	0	6	13,6	3	0	3	3,9
Equus burchelli	19	2	21	3,4	1	2	3	6,8	2	1	3	3,9
Kobus ellipsiprymnus					1	0	1	2,3				
Connochaetes taurinus	12	4	16	2,6	1	1	2	4,6				
Tragelaphus strepsiceros	8	5	13	2,1	3	0	3	6,8	1	1	2	2,5
Tragelaphus angasii									2	0	2	2,5
Tragelaphus scriptus	2	1	3	0,5					1	0	1	1,2
Aepyceros melampus	62	23	85	13,6	3	0	3	6,8	18	5	23	28,8
Phocochoerus aethiopicus									3	1	4	5,0
Potamochoerus porcus									1	0	1	1,2
Raphicerus campestris									0	1	1	1,2
Crocuta crocuta	1	0	1	0,2								
Carnivora									2	0	2	2,5
Crocodylus niloticus					2	2	4	9,1				
Unidentified chip	69	381	450	71,9	5	4	9	20,5	24	14	38	47,5
Total	205	421	626		30	14	44		54	19	80	
Percent	33	67			68	32			70	30		
Porcupine quills			156				409				113	
% Porcupine gnawed				1,3				13,6				15,0
% Skull bones and horns*				18,8				20,0				14,3
% Leg bones and hooves*				71,0				45,7				59,5
% Vertebrae, ribs & pelvis*				10,2				34,3				26,2

*Unidentified chips were excluded in these calculations.

Table 4 Items carried to den Site 6 during one month and the remains found inside and within 10 m of the den at the end of this period

Date	Species	Part	Remains	Pieces
08/06/84	Kudu	back leg	none	
10/06/84	Buffalo	front leg	hoof-radius	1
10/06/84	Buffalo	back leg	hoof-tibia	1
17/06/84	Zebra adult	front leg	hoof-radius	1
18/06/84	Zebra adult	front leg	none	
27/06/84	Zebra foal	hindquarters	hooves-vertebrae	29
30/06/84 –	Impala	back legs	metatarsals	2
07/07/84	Kudu	front leg	hoof-scapula	1
	Kudu	front leg	hoof-scapula	1
Total				36

Transport of animal remains

Of 134 carcass pieces, comprising soft parts (29,2%), long bones (55,9%), vertebrae and skulls (14,9%), which were observed to be carried away from carcass sites by spotted hyaenas, 12,7% were taken further than 1 km (Table 5). Usually this had the effect of reducing interference competition for available food, as pieces were removed to be consumed at leisure in relative safety and peace. On 18 occasions such items were cached at different sites, usually in grass clumps under bushes and once in water. Eleven cached items were later observed to be retrieved. This behaviour of spotted hyaenas usually had the effect of dispersing animal remains, but because it was neccessary for a female with cubs to spend much time at the den, she sometimes took food items there rather than to a different place, with the result that bones could accumulate at a den site, but nowhere else.

Discussion

The bone accumulations found in the Kruger National Park support previous findings that spotted hyaenas may sometimes play an important role as agents in the establishment of bone assemblages. Porcupines cannot be precluded as possible agents that could have contributed to some of the collections. However, they could not have played a major role, because of the low proportion of bones with gnaw marks, and the large size of many of the bones at the dens. In contrast, the type of damage seen on many of the bones was characteristic of the splintering, gnawing and partial digestion (prior to
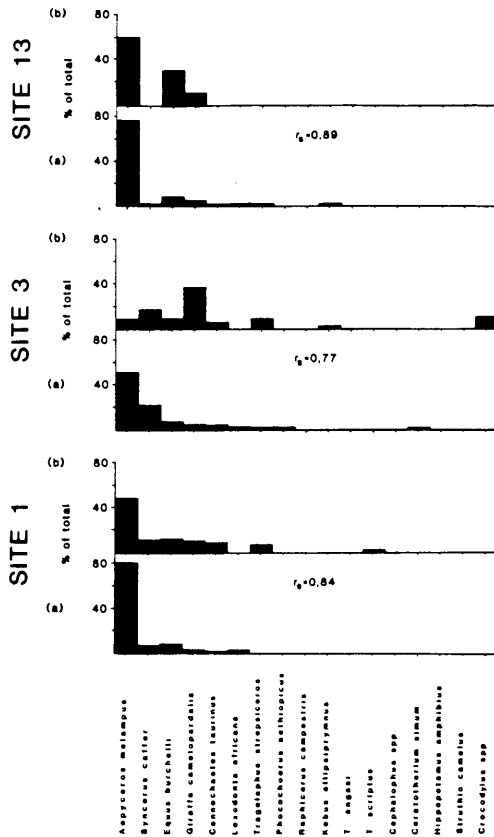
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Figure 3 Histogram depicting the relative abundance of prey species: (a) counted in the immediate vicinities (31,5 – 50,4 km²) of den sites during six successive annual aerial censuses (1979 – 1984) and (b) represented in bone accumulations at den sites.

Table 5 Transport of bones by spotted hyaenas away from carcass sites

Distance	Frequency	Percent
< 100 m	65	48,5
100 – 1 000 m	52	38,8
1 – 2 km	10	7,5
2 – 6 km	7	5,2
Total	134	100,0

regurgitating), caused by spotted hyaenas (Sutcliffe 1970). Furthermore, the preponderance of long bones can be explained from direct observations of spotted hyaena behaviour in the present study, and compares well with previous studies of spotted hyaena assemblages (Henschel et al. 1979; Hill 1980b).

Casual observations at culverts and earthen dens indicated that these were seldom used for a long period of time. On the other hand, granite and calcrete caves provide more permanent protection, have existed much longer than man-

made structures, such as roads with culverts, and are repeatedly used. It is therefore not surprising to find that most bones accumulated at granite or calcrete caves. We have no idea over what time-span the 626 bones at Den 1 were accumulated. However, judging from their texture and odour, many of the bones were of very recent origin.

The debate concerning the role of spotted hyaenas as bone-collecting agents was a result of the facultative nature of their food transporting behaviour. Unlike Hyaena hyaena and H. brunnea, Crocuta crocuta does not provide its cubs with meat (Kruuk 1972), except for one observation recorded by Hill (1980a). However, we observed hyaenas moving food frequently from one locality to another as a means of avoiding interference competition. This can sometimes result in an accumulation forming at a den, depending on the inclinations of certain individuals. In a similar way, the close proximity of a human settlement could be a disturbance that leads hyaenas to carry food remains to their lairs, as was described by Sutcliffe (1970) in an East African region.

The examination of various den sites by different authors has contributed to differing interpretations of the role of Crocuta as bone-accumulating agents. Some of the dens we examined had quite large bone accumulations, similar to dens examined by previous authors (Mills & Mills 1977; Henschel et al. 1979; Hill 1980b), while others had few or no bones, similar to dens examined by Hughes (1954; 1961). However, the number of bones found in Crocuta assemblages does not compare to the large number found at Hyaena dens in Israel (Skinner et al. 1980) or Namibia (Skinner & van Aarde in press). Both the latter species collected a larger proportion of bones of smaller prey species and Crocuta assemblages contained a greater proportion of bone chips, reflecting the more powerful crushing ability of Crocuta jaws.

Conclusion

Four types of dens are used by spotted hyaenas in the Kruger National Park. The more permanent granite and calcrete caves could serve many generations of hyaenas, while aardvark holes and road culverts are not only more temporary, but the bones are exposed to more weathering. Assemblages are formed on a facultative basis by Crocuta and reflect their tendency to move food away from carcass sites. The assemblages thus formed show a predominance of long bones over vertebrae, the representation of species resembling the local ungulate populations. These results support the validity of describing ancient medium-to-large ungulate fauna from an analysis of ancient hyaena dens.

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Appendix Skeletal parts identified at Dens 1, 3 and 5.
1 = horn; 2 = skull; 3 = maxilla; 4 = mandible; 5 = tooth; 6 = vertebra; 7 = pelvis; 8 = rib; 9 = scapula; 10 = tubercle; 11 = humerus; 12 = radius/ulna; 13 = femur; 14 = tibia/fibula; 15 = astralagus; 16 = metatarsal/metacarpal; 17 = phalange; 18 = hoof.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Den 1																		
Giraffe		1	3		2					1	1	2	1	2	1	4		
Buffalo						1	2		1		1	3	3	4	1	1		2
Zebra				1	6	1						1		3		7		2
Wildebeest	3					2	2				1	2	2	1		3		
Kudu			2									3		2	1	2		3
Bushbuck								1			1					1		
Impala	12	1		6	1		3		4	1	5	11	1	6		18		16
Hyaena					1													
Den 3																		
Giraffe						4	3		2	1	1			2				
Buffalo						2	1		2		1							
Zebra											1	1		1				
Waterbuck											1							
Wildebeest														1		1		
Kudu	1						2											
Impala	2								1									
Crocodile			2	2														
Den 5																		
Buffalo						1		1						1				
Zebra						1					1							1
Kudu												2						
Nyala														1		1		
Bushbuck																1		
Impala	1	2		1		5	2	2			2	2		2	1	3		
Warthog				1	1							1				1		
Bushpig																1		
Steenbok									1									
Carnivore														1			1	

Improved age estimation in spotted hyaenas (*Crocuta crocuta*)

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Introduction

Reliable age estimation of spotted hyaenas (*Crocuta crocuta*) has proved frustrating. Kruuk (1972) distinguished between five tooth-wear classes using the third mandibular premolar (PM_3), and extrapolated these to absolute ages (years) by assuming a constant annual mortality rate for the construction of life tables. Lindeque & Skinner (1984) enlarged on this approach and defined seven age classes using size frequency analysis of the occlusal surface of PM_3.

In order to provide a technique allowing for age classes with equal intervals which could be used for comparative analysis of different populations, the occurrence of dentine lines or annuli was investigated in spotted hyaenas from the Transvaal Lowveld. While dentine lines are strongly correlated with absolute age in all investigated carnivores (Driscoll, Jones & Nichy, 1985), the extraction of teeth from live carnivores for age determination purposes is seldom practical. This led us to investigate whether non-destructive parameters of tooth wear might correlate with dentine lines. We find that the parameters of PM_3, surface area and tooth height provide useful indices of age for future field work.

Materials and methods

A total of 57 spotted hyaena skulls and dental impressions (23 males, 28 females and 6 sex unknown) provided the data for this study. Some of these skulls ($n = 32$) had been used previously by Lindeque & Skinner (1984). Seven skulls and 9 dental impressions, obtained from immobilized animals, were collected in the Kruger National Park from 1983–1985. In addition, 9 skulls collected in the Transvaal Lowveld were available from the Transvaal Museum.

Dental impressions: Dental impressions from PM_3 of immobilized animals ($n = 7$) were made, using an elastic impression compound (Alginate CA 37: Keurs & Snetjes Dental Mfg Co., Haarlem, Holland) placed in stainless steel dental trays. The impressions were kept moist until reverse casts could be made with plaster of paris (Herosa: Plastoria Distributors (Pty) Ltd., Pretoria, S.A.). In a control experiment, measurements of the surface areas of the PM_3 (see below) of hyaena skulls ($n = 6$) of various ages were compared to plaster casts of the same teeth.

Occlusal surface area of PM_3: On both sides of the jaw ($n = 57$) surface areas of teeth were measured according to the technique devised by Lindeque & Skinner (1984), with the following modifications: magnification was standardized by prefocusing a stereo-microscope (Wild M5, fitted with a drawing tube) on an arbitrary focal point, placing a scale (calipers) at this point and drawing the superimposed image. All the surface areas were measured by the same observer, who raised the occlusal surfaces into the predetermined focal point, and drew the areas on to cardboard. The area of the outline drawing was calculated as the mean value of 3 consecutive readings with a planimeter (KP-21 Koizumi compensating planimeter, Hayashi Denkoh Co. Ltd., Tokyo). The mean value of the 2 ipsilateral teeth was used as an estimate of wear. Ten consecutive measurements of a single outline drawing revealed a high consistency (coefficient of variation $< 0.1\%$).

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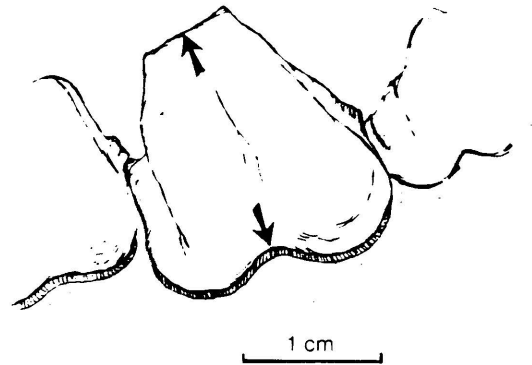
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1 cm

FIG. 1. A diagram illustrating the measurement of PM₃ height in the spotted hyaena.

Height of P.M₃: Height of the third mandibular premolars was determined by using vernier calipers (see Fig. 1). The mean value of the 2 ipsilateral teeth was used for analysis.

Dentine annuli: Mandibular canines (22 right and 6 left) were removed from 25 hyaena skulls. The undecalcified teeth were mounted on perspex blocks using polyester and bissected longitudinally with a modified, single blade, Beuhler Isomet low-speed saw. The bisected teeth were then ground (Sarrazin & Ouellet, 1978), polished (Laws, 1952), etched and stained following Mattlin (1978). Dentine annuli were
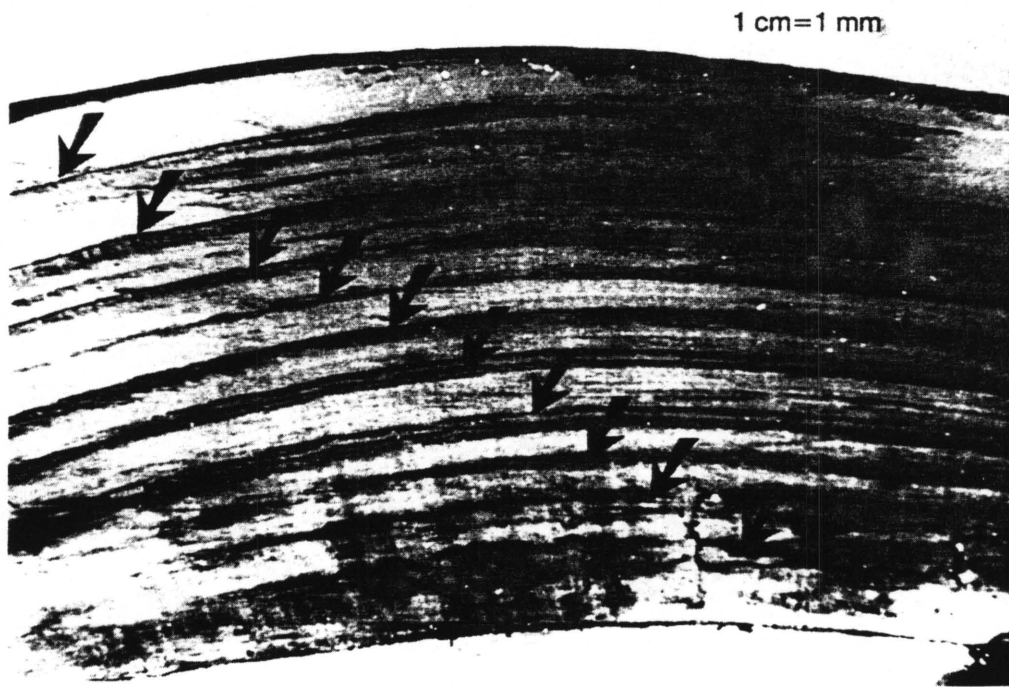
1 cm = 1 mm

PLATE I. A longitudinal section of a spotted hyaena canine (11 years old). Arrows indicate dentine lines shown up by lightly rubbing an etched tooth with carbon paper.
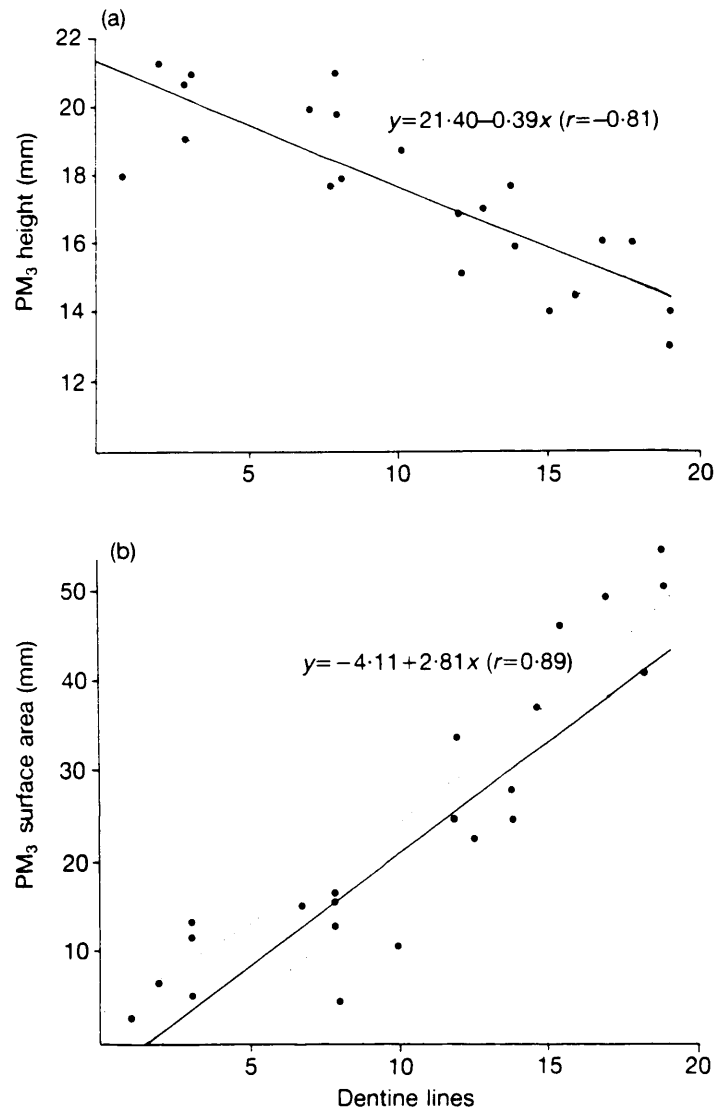
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FIG. 2. Relationships and 95% confidence intervals for the regression analyses between: (a) number of dentine lines and PM₃ height ($n = 22$), and (b) number of dentine lines and PM₃ surface area ($n = 22$).

counted by 3 observers using reflected light. Counts were done with or without rubbing the tooth surface lightly with carbon paper (see Plate I).

Tooth eruption: Tooth eruption patterns were monitored for 3 known-age cubs kept in captivity.

Statistical analyses: Comparisons between ipsilateral pairs were made using the Wilcoxon matched-pairs signed test; differences between means were assessed using Student's t-test. Coefficients of variation (C.V.) were calculated and linear or multiple linear regression analyses were carried out using SAS software (Statistical Analysis System Institute Inc., Carey, N.C., USA). Due to the absence or breakage of some teeth,

all variables could not be measured from all skulls and the final sample sizes included in each analysis are indicated where appropriate.

Although the present findings are subject to confirmation following analysis of known-age or tetracycline-treated material, they are applicable as any relative error made during the present analysis can be compensated for later.

Results

In the control experiment, the measurements of surface areas from skulls did not differ significantly ($t_5 = 1.92$; $P > 0.1$) from plaster casts of the same teeth. The coefficient of variation for occlusal surface areas of PM_3 between ipsilateral teeth was 15.09%, with the left side being significantly more worn than the right side ($T_s = 106.11$; $P < 0.005$; $n = 47$). For tooth height, no constant difference in wear between the left and right sides ($n = 40$) was evident (C.V. = 2.87%). The expected relationship between height (independent variable) and surface area (dependent variable) of PM_3 was best described by the equation $y = 135.17 - 6.39x$ ($r = 0.87$; $n = 45$).

Deciduous incisors and canines were erupted in two cubs at four days of age and the first signs of permanent teeth (incisors) were observed at seven months of age ($n = 3$). Permanent canines erupt at an age of 12 months and are fully erupted at 15 months (M.G.L. Mills, pers. comm.).

Dentine and von Ebner lines were visible in all teeth examined (see Plate I). Although counts were more difficult in older individuals, no teeth with closed pulp cavities were found. Disagreement in counts by the observers varied from 0–3 rings per tooth (mean difference = 0.83; $n = 28$), with the greater difference (> 2) occurring in four older individuals (> 18 lines). Mean values were taken as the correct counts. As the maximal difference between ipsilateral teeth ($n = 3$) was one, only a single tooth from each skull was included in the final regression analyses.

The respective positive and negative relationships found between PM_3, surface area and height, with the number of dentine lines are described best by the regressions shown in Fig. 2. The analyses show that the number of lines in teeth may be estimated by using either PM_3 height ($r = -0.81$; $n = 22$) or surface area ($r = 0.89$; $n = 22$), with surface area giving the most reliable prediction. The regression analysis of dentine lines against volume (area × height) failed to increase the correlation ($r = 0.87$; $n = 22$). However, the reliability of prediction could be marginally increased when both PM_3 height and surface area were analysed in a multilinear model (Mallow's statistic $C_p = 3.00$ cf. 1.50 and 15.88 for PM_3 surface area and height, respectively; $r = 0.89$; $n = 22$).

Discussion

Limitations in Kruuk's (1972) technique, and Lindeque & Skinner's (1984) expansion of it, restricts potential population and reproductive studies. Moreover, spotted hyaenas breed aseasonally (Lindeque & Skinner, 1982), which means that all samples analysed for multimodality of size frequency distribution would not necessarily reveal a large number of age-cum-size cohorts (Cassie, 1954). For example, the sample used in the present analysis revealed only three such age classes. In addition, cementum line counts in this species are inconsistent (unpubl. obs.), although these were used by Frank (1983) as indicators of relative age.

The present study reveals evidence of dentine growth lines in the permanent canines of spotted hyaenas. Such dentine lines have been used as indicators of absolute age in several carnivore species (Driscoll, Jones & Nichy, 1985). The similarity between the maximal number of dentine rings found in the present study and the maximal longevity recorded for spotted hyaenas (20 lines

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cf. 18–25 years in captivity; see Kruuk, 1972) provides circumstantial evidence that dentine rings are formed annually and could be indicative of absolute age. Permanent canines erupt between 12 and 15 months and, if the observed dentine lines are annuli, it is likely that the first dentine line is deposited between 12 and 24 months of age. This predicts a discrepancy of at least one year between the age and number of dentine lines in *Crocuta*.

To conclude, the good correlation between indices of tooth wear and occurrence of dentine lines in spotted hyaenas indicates that either of them can be used as estimates of absolute age.

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Spotted hyaena monomorphism: an adaptive 'phallusy'?

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Prevailing hypotheses concerning the evolution of sexual monomorphism in the spotted hyaena, Crocuta crocuta, are critically examined and found to contain logical and empirical inconsistencies. Instead, an alternative hypothesis is advanced based on genetic translocation from the Y to the X chromosome. This would have exposed the female foetus to raised levels of testosterone and led to social dominance by the adult female.

Die gangbare hipoteses oor die evolusie van geslagsmonomorfisme by die gevlekte hiëna, Crocuta crocuta, is krities geëvalueer. 'n Aantal logiese en empiriese teenstrydighede is hierby opgemerk. 'n Alternatiewe hipotese word nou op grond van 'n genetiese translokering van die Y- na die X-chromosome voorgelê. As gevolg hiervan sou die vroulike fetus aan verhoogde testosteroonvlakke blootgestel gewees het, wat tot sosiale oorheersing deur die volgroeide wyfie gelei het.

In no single group of animals is the existence of separate sexes more obvious to human perception than in the mammals. It is therefore hardly surprising that any mammalian species in which this difference is conspicuous by its absence would become the subject of extensive biological enquiry. Biology has only one unifying concept with which to explain biological form and function, in the theory of adaptation by natural selection, as proposed by Darwin and Wallace.[1] It was thus inevitable that biologists would try to explain the lack of sexual dimorphism in a particular mammal in terms of the possible adaptive advantages that such a feature could offer. The animal referred to is the spotted hyaena, *Crocuta crocuta*, the subject of numerous fascinating and unjustified myths,[2] some of which led to their being referred to as hybrids and hermaphrodites.

It is the aim of this paper to show how the assumptions that natural selection is an optimizing process, and that sexual monomorphism must be the result of some selective advantage directly associated with the morphology of the external genitalia,

have led to the proposal of unacceptable, teleological hypotheses concerning the origin of monomorphism in this species. The inclusion of this paper in this symposium should therefore strike a cautionary note; this example, taken from the biological literature, illustrates the misconceptions that can arise when the topic under investigation becomes subject to unjustified extrapolations from disciplines that are essentially non-historical, such as embryology and ethology.

Existing hypotheses

A number of hypotheses for the evolution of genital monomorphism in the spotted hyaena have been proposed:

1. The peniform clitoris enables members of the same sex to afford sexual gratification to each other during the rut.[3]
2. The peniform clitoris evolved in females for use during the elaborate meeting ceremony performed by this species.[4]
3. The peniform clitoris has evolved as an organ for signalling social status.[5]
4. The peniform clitoris is an incidental consequence of the action of high blood androgen levels upon sexual development in the foetus.[6,7]

The first three hypotheses all have an ethological origin and are based on the greeting ceremony, a unique feature of this species. The fourth explains the occurrence of sexual monomorphism in terms of the developmental pattern found in *Crocuta*. In order to test the validity of these hypotheses, a critical evaluation of the available empirical evidence is required. Of the three ethologically based postulates, only the third[5] offers an evolutionary scenario which can be critically evaluated. The Hamilton *et al.* hypothesis can be summarized as follows:

. . . effective female competition with males at carcasses was advantageous. . . . Selection for increased female body size, a major determinant of rank, was accompanied by selection for increased female aggression, mediated by increased androgen levels. Changes in female hormone levels could lead to initial virilization of the female external genitalia; these rudiments were incorporated into a novel genital display — or possibly one already occurring in males — leading to further selection for enlarged and fully erectile phalluses in females.[5]

The first step in this thesis is an increase in female body size accompanied by raised female aggression, mediated by elevated androgen levels. This could easily become a circular argument as their data showed that females are heavier, but not necessarily larger limbed, than males. This Hamilton *et al.* considered to be no more than a reflection of the animals' social status and their priority access to food. By suggesting a simultaneous increase in body size, aggression and androgen levels, these authors are subtly circumventing a 'chicken and egg' problem. If larger female body mass is indeed a post facto reflection of preferential access to food, then we see no other vehicle for the establishment of female dominance than an initial increase in aggression. Just how this greater female aggression arose is the subject of our alternative hypothesis.

The second assumption of the Hamilton *et al.*[5] hypothesis is that increases in adult female androgens resulted in more aggression. If this were so one might expect to find relatively higher levels of the hormone that mediates aggression, namely testosterone,[8] in female spotted hyaenas compared to other female mammals. The only available data on the peripheral testosterone levels of spotted hyaenas, based on assays of an acceptable accuracy, sensitivity and specificity, are given by Racey and Skinner,[6] Gombe,[9] and Lindeque *et al.*[10] The study by Frank *et al.*[11] measured total androgens, rendering their results incomparable with those obtained in the other studies. The relative androgen levels for the three hyaena species as reported by Racey and Skinner[6] are shown in Fig. 1. It is apparent that female spotted hyaenas do not have high testosterone or androstenedione levels, but the males show low concentrations. These findings are supported by Gombe[9] and Lindeque *et al.*[10] The latter found male testosterone levels ranging from 0,2 – 1,6 ng/ml, and female values ranging from 0,2 – 3,6 ng/ml. These equivalent testosterone levels found in both males and females may have resulted from social tensions, as prolonged psychological stress alone may suppress peripheral testosterone concentrations.[11] However, Racey and Skinner[6] found significantly higher testosterone levels

Fig. 1. Histogram depicting the mean (± s.d.) peripheral testosterone (□) and androstenedione (▥) titres found in the different sexes of the three hyaena species: *Crocuta crocuta, Hyaena brunnea* (brown hyaena) and *Hyaena hyaena* (striped hyaena). Sample sizes are given at top of columns. Data from Racey and Skinner.[6]

in testes than in ovaries, providing a further indication that the similar peripheral testosterone values in adult males and females may be solely a result of social stress, or of relative social status. These data do not suggest that the females have elevated testosterone, indicating that a cause other than an increase in this hormone level in the adult is responsible for the establishment of raised female aggression.

Thirdly, Hamilton *et al.*[5] suggest a gradual evolution of the external genitalia in the female, following an initial virilization due to high female androgen levels. This part of their hypothesis is incompatible with established theories of sexual differentiation in mammals. It implies that the development of masculinized external genitalia in the normal, male foetus of mammals is not solely a consequence of prenatal exposure to testosterone, as proposed by Jost.[13,14] The timing of prenatal exposure to this androgen in mammals is critical for the effective development of masculinized external genitalia.[15] There is no evidence to show that an identical foetal testosterone exposure would result in differential virilization between the sexes, provided the extent and the timing of the hormonal secretion corresponded with the natural male peak. In other words, embryologically speaking, the external male and female organs should be seen as homologous. The similar testosterone profiles found in *Crocuta* foetuses of both sexes by Lindeque and Skinner[16] provides support for this view (see Fig. 2). The proposed embryological hypothesis[6,7] is, therefore, more consistent with the available empirical evidence. However, one further problem remains, which is to explain why the Wolffian ducts in the foetal female were unaffected by this testosterone exposure of the foetus.

The demonstration that the appearance of the male genitalia is a result of a particularly interesting development does not, however, solve the question of its evolution. Apparently the spotted hyaena has been subject to some special selective pressure, which we propose was for increased aggression in the adult female, resulting from prenatal testosterone exposure, and that an additional consequence of this phenomenon was the development of a male appearance.

The three complementary ethologically based hypotheses, of which the postulate by Hamilton *et al.*[5] is the most recent, contain a number of logical and empirical inconsistencies that have to be overcome if any are to gain acceptance, whereas the embryological postulate seems to conform more closely to the available evidence. However, how far do any of these proposals go towards explaining sexual monomorphism in this species? We will argue that all these hypotheses share a further flaw that is more serious than a simple lack of conformity with empirical evidence.

Historical functionalism

It is generally accepted that any biological phenomenon can be investigated from either a historical or a non-historical perspective, and an investigation into the origins of sexual monomorphism in the spotted hyaena is inherently historical. On the other hand, the disciplines used to formulate the four hypotheses (ethology and embryology) are essentially of a non-historical nature,[17] as they are primarily concerned with the manifest properties of organisms and not with how those properties or ontogenies became established.

All four hypotheses stress current function (for example, coitus, meeting ceremony, signalling social status, testosterone-mediated dominance). The ethological hypotheses stress this directly, whereas the embryological approach invoked some ultimate selective pressure, again related to current functions (greeting ceremony and social dominance).[6,7] An inability to find any mechanistic reasons for the evolution of function resulted in the extension of teleological causality, or non-historical studies, into the historical realm. This approach led to expressions such as: this performs a function, therefore it must have originated for that reason. Such statements which stress current function are
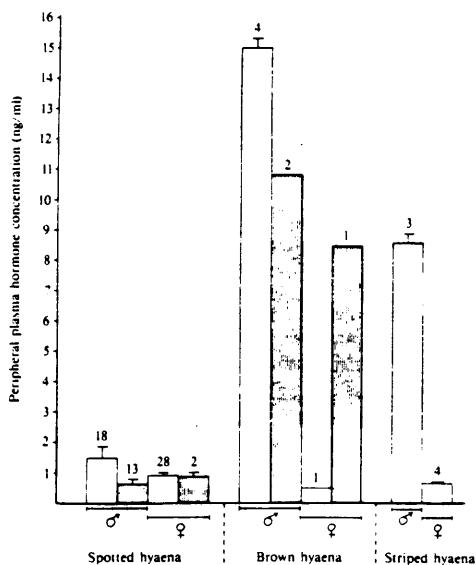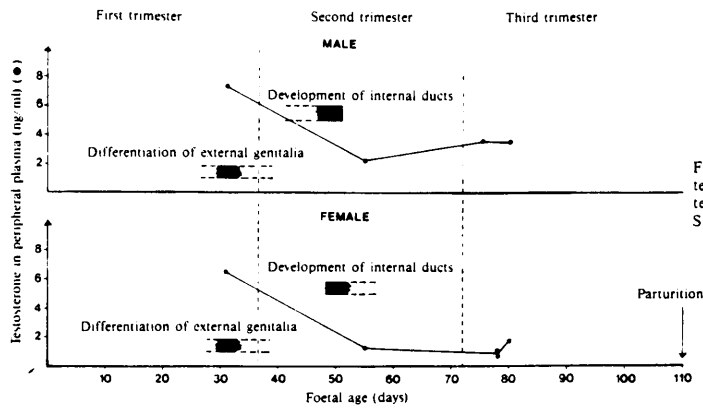
Fig. 2. A schematic representation of peripheral testosterone titres in both male and female spotted hyaena foetuses. Data from Lindeque and Skinner.[16]

unacceptable teleological (goal-seeking) claims, as they amount to historical functionalism.[17] The only difference between the ethological and the embryological hypotheses is that the latter reduces the role played by the peniform clitoris in the meeting ceremony from an adaptive to an exaptive phenomenon.[1] Thus, we still have no single evolutionarily sound hypothesis to explain the evolution of sexual monomorphism in the spotted hyaena.

An alternative, non-teleological hypothesis

An investigation of the embryological origins of sexual monomorphism in the spotted hyaena has revealed that foetal secretion of large quantities of testosterone during the phenotypic development phase causes the masculinization of the female's external genitalia.[6,18] This results in an immature female with a very narrow clitoridal meatus, which makes copulation impossible. In the oestrous or parous female the meatus does, however, enlarge sufficiently to ensure that the clitoris does not hinder penile intromission or parturition.[18,19] This enlargement is probably due to increased oestrogen and progesterone secretion at puberty.[20,21] One can thus appreciate that the evolution of genital monomorphism in this species took place without being an impediment to the mechanics of reproduction.

Before the onset of steroid synthesis in mammals, the primordial gonads of both sexes have the capacity to perform all reactions involved in steroid synthesis, except for two (see Fig. 3), namely the cleavage of cholesterol to pregnenolone (the precursor of all steroid hormones) and 3β-hydroxysteroid dehydrogenase activity. The enzymes required for the remaining three reactions in the conversion of cholesterol to testosterone are abundant in

both gonads. At the time of elevated testosterone synthesis in the foetal testis, there is an increase of pregnenolone formation in both foetal testis and ovary. This is coupled with a simultaneous increase of 3β-hydroxysteroid dehydrogenase activity in the Leydig cells of the testis only.[15,23] In contrast, in all species investigated to date,[23] the maturation of 3β-hydroxysteroid dehydrogenase activity starts only towards the end of gestation in the foetal ovary. The presence of this sex-related difference in enzymatic activity leads us to suggest that the maturation of 3β-hydroxysteroid dehydrogenase activity is related to the Y chromosome. Testosterone secretion by the foetal female hyaena is facilitated by the general pattern of gonadal differentiation in carnivores,[22] which results in the presence of large quantities of interstitial tissue in the foetal ovary during phenotypic development. A reduction of this testosterone-producing interstitial tissue occurs towards the end of gestation.[16,24]

Implications of prenatal testosterone exposure

The prenatal exposure to testosterone has an additional effect, besides the stimulation of the male-type external genitalia, and that is it results in a more aggressive individual.[25] Furthermore, there is clear evidence that a genetic component is involved in the control and variation of aggressive behaviour in mammals.[26] Shrenker and Maxon's study,[27] using back-cross breeding experiments, revealed that differences in aggressive performance in mice (*Mus musculus*) were attributable to a combination of effects stimulated by the Y chromosome and autosomal suppression, probably resulting from differences in testosterone secretion.[4] If, as Jost[13,14] proposed, chromosomal sex directs the development
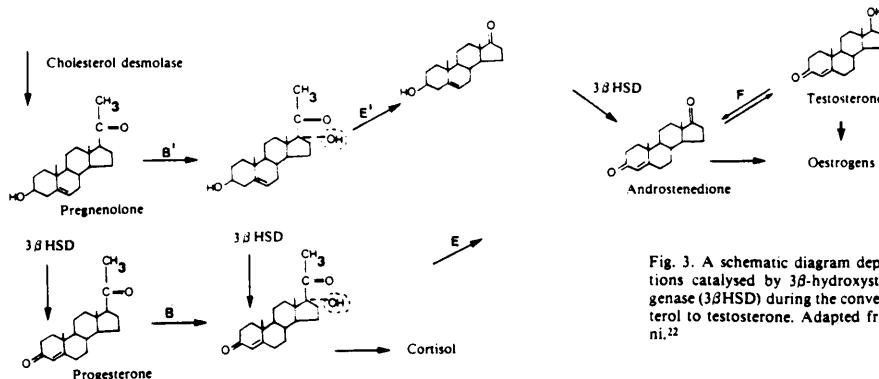

Fig. 3. A schematic diagram depicting the reactions catalysed by 3β-hydroxysteroid dehydrogenase (3βHSD) during the conversion of cholesterol to testosterone. Adapted from Bangiovanni.[22]

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of phenotypic sex, the chromosomes should also direct prenatal testosterone secretion in the foetal males of mammals. A translocation of the segment of the Y chromosome responsible for this role, or its regulator, to an autosome or X chromosome could explain the occurrence of a male-type increase in 3β-hydroxysteroid dehydrogenase activity. Similar translocations, with varying overlapping regions, have been reported in males with apparently normal 46,XX karyotypes.[28] This would result in prenatal exposure to testosterone in the female hyaena foetus during phenotypic development when interstitial tissue is available in the ovary. This exposure to testosterone would in turn lead to increased adult female aggression.[25]

It is thus assumed that the gene for aggression in the Y chromosome, or its regulator, which originally was present only in male spotted hyaenas, would also occur in females after gene translocation. If this were so, it would be an unusual record of how a laterally spreading gene has influenced mammalian morphology. Confirmation of this hypothesis awaits the mapping of the 3β-hydroxysteroid dehydrogenase gene in mammals, using recombinant DNA techniques.

Sociality

The spotted hyaena's social unit consists of a nucleus of closely related resident females.[29,30] Natal males disperse at maturity whereas non-related males join the clan to mate.[4,29,30] The females' relationships[31] are characteristic of a female-bonded group.[32] Increased aggression could conceivably result in increased dominance[33] and raised reproductive success. Thus, our proposed selection pressure for adult female aggression would facilitate the establishment of female dominance[4,11,30] in spotted hyaenas, as it would result from a combination of increased female aggression and cooperation between closely related females to outcompete unrelated immigrating males. It is unknown whether female philopatry existed prior to the gene translocation discussed above, or whether it is a consequence of increased

aggression, which made group hunting possible.

Our hypothesis proposes that genital monomorphism in *Crocuta* arose as a consequence of a punctuated genetic translocation, which produced exposure to testosterone in the female foetus and pronounced aggression in the adult female. We further propose that this could have led to the establishment of female dominance in this species, owing to the male-biased dispersal strategy. This hypothesis in effect suggests that the evolution of sexual monomorphism and the establishment of female dominance were related events. Furthermore, we imply that the male sexual facies found in female spotted hyaenas arose as a pleiotropic effect of a genetic translocation, and that it was a non-adaptive consequence of foetal exposure to testosterone. The peniform clitoris and false scrotum are therefore seen as exaptations incorporated into social communication among hyaenas, as suggested by Gould and Vrba,[7] rather than a case of 'sexual mimicry'. This hypothesis also supports the proposal that the greeting ceremony is instrumental in enhancing present-day mutualistic relationships.[30]

Conclusion

Our discussion of the prevailing hypotheses on the evolution of sexual monomorphism in the spotted hyaena reveals the inherent dangers of invoking current function when evolutionary causes for biological structures are sought. It is essential that the true nature of teleological statements be recognised, especially when they are applied in a historical perspective. Biologists cannot assume that natural selection is an optimizing process. Biological structures evolve and a search for the adaptive value of some of them may be in vain.

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Short communications

The use of Zoletil for the immobilization of spotted hyaenas

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The use of Zoletil for the chemical immobilization of spotted hyaenas Crocuta crocuta is described. A dose of 250 mg/ individual ca 4 mg/kg) was found effective for the immobiliza- tion of wild adult spotted hyaenas, whereas this dosage could be reduced to 2 mg/kg for captive individuals. The drug was used on hyaenas of a wide range of ages and on females in an advanced stage of pregnancy without any adverse effects.

Die gebruik van Zoletil vir die chemiese immobilisasie van gevlekte hiënas word beskryf. 'n Dosis van 250 mg/individu (ca 4 mg/kg) was effektief vir die immobilisasie van wilde volwasse gevlekte hiënas terwyl die dosis tot 2 mg/kg vir hiënas in gevangenskap verlaag kon word. Die middel is sonder enige komplikasies op hiënas van 'n wye reeks ouder- domme asook op wyfies in 'n gevorderde stadium van drag- tigheid gebruik.

Keywords: CI-744, Crocuta crocuta, immobilization, Hyaenidae, Zoletil

Zoletil (CI-744: Anchorpharm (Pty) Ltd, Bramley) is a 1 : 1 combination of zolazepam hydrochloride (CI-716) and tiletamine hydrochloride (CI-634). When adminis- tered alone tiletamine HCl produces a cataleptoid anaesthesia with profound analgesia which is often accompanied by convulsive seizures and clonic or tonic muscular reactions in cats (Gray, Bush & Beck 1974). Zolazepam HCl, on the other hand, is a diazepinone tranquilizer which can cause belligerency when used alone. When combined, the side effects are eliminated and the combination acts as a potent dissociative immobilization agent (Boever, Holden & Kane 1977).

This drug which is presently only available for research purposes in South Africa, has been used successfully for the immobilization of birds, reptiles and mammals. Published data for very limited sample sizes of 180 species, including the problematic primates (51 species) and carnivores (50 species) are available (Gray, Bush & Beck 1974; Eads 1976). These preliminary investigations were aimed at establishing the range of species for which the drug could be useful and very few intensive reports concerning the action of this drug on specific species are available. Among the carnivores the drug has been found suitable for immobilizing tigers *Panthera tigris*, leopards *Panthera pardus* and lion *Panthera leo* using doses of 4–5 mg/kg (Seidensticker,

Man Tamang & Gray 1974; King, Bertram & Hamilton 1977).

Spotted hyaenas, *Crocuta crocuta*, have been immobilized with a number of chemical immobilizing agents including succinylcholine chloride, phencyclidine hydrochloride, ketamine hydrochloride and etorphine hydrochloride (see Kruuk 1972; Ebedes 1973; Smuts 1973; Harthoorn 1976; Van Jaarsveld, McKenzie & Meltzer 1984). The advantages and disadvantages associated with the use of these drugs have been discussed by Smuts (1973), Lindeque (1981) and Van Jaarsveld, McKenzie & Meltzer (1984) respectively. This communication reports on the effectiveness of the chemical immobilization agent, Zoletil, for the immobilization of spotted hyaenas.

Twenty spotted hyaenas of various ages (4 days–20 years) were subjected to 39 immobilizations with Zoletil in the Johannesburg Zoological Gardens (n = 1), National Zoological Gardens (n = 16), Kruger National Park (n = 6), Kalahari Gemsbok National Park (n = 9) and the Experimental Farm of the University of Pretoria (n = 7). The hyaenas were darted with Telinject equipment (Telinject S.A., Randburg) or injected with a syringe. Adults were immobilized with a standard dart containing 250 mg Zoletil, whereas attempts were made to administer doses of approximately 4 mg/kg to juveniles. Following immobilization all animals were treated according to the clinical procedures described in Van Jaarsveld, McKenzie & Meltzer (1984). Seven animals were also subjected to extended surgical anaesthesia with halothane following immobilization (see Van Jaarsveld, McKenzie & Meltzer 1984).

Mean values and ranges of mass, administered dose, time to ataxia, recumbency and recovery are given in Table 1. The drug does not cause any excitement during induction which results in a tranquil immobilization. Excessive salivation frequently occurs and the use of atropine is suggested if an animal is going to be recumbent for any length of time. Although atropine has not been used on spotted hyaenas to date, preliminary studies have indicated that tiletamine HCl (CI-634) plus atropine may not only be compatible but possibly even be synergistic (Gray, Bush & Beck 1974). Excessive salivation was, however, not problematic in the present study as both the pharyngeal and laryngeal reflexes were present. The presence of these reflexes did not prevent intubation for inhalation anaesthesia. All palpebral, pinnal and pedal reflexes remained functional and, owing to the dissociative nature of the drug, attempts to monitor the plane of anaesthesia using these reflexes

Table 1 Results of 39 spotted hyaena immobilizations using Zoletil administered intra-muscularly

	Mass (kg)	Darting dose (mg/kg)	Ataxia (min:s)	Recumbent (min:s)
n	39	39	11	11
Mean ± SD	30,91 ± 23,85	3,45 ± 2,04	2:15 ± 1:10	2:40 ± 1:53
Range	00,90–81,00	2:00–12:00	00:56–5:00	1:05–8:30

were not satisfactory. At high doses (± 8 mg/kg) a state of surgical anaesthesia is obtained.

The recovery was generally very smooth although some 'head flopping' did occur. The short immobilization period (75 min–120 min) after darting makes this drug ideal for general physiological sampling and the fitting of radio collars. The fact that the animal is generally completely mobile within 2 h after darting is time saving for field workers compared with the recovery time for drugs such as phencyclidine HCl. The recovery time after extensive halothane anaesthesia (4 h) was extended and the animals were mobile between 90 and 240 min. These results indicate that Zoletil can safely be used on hyaenas of all ages. Three individuals in an advanced stage of pregnancy were also immobilized without any notable adverse effects. Because of the smooth immobilization, rapid recovery, wide ranges of ages and physiological conditions and the lack of mortality, together with the relatively wide safety margin of this drug (see Table 1), its use is recommended.

Zoletil is supplied in a freeze-dried form and the manufacturer's recommendation that the drug should be used within 24 h after reconstitution should be adhered to because the drug loses its potency after this. Zoletil was also found to keep better at 4°C once dissolved. A reduction in immobilizing potency was accompanied by a slight discolouration from transparent to light brown. This does, however, not pose a serious problem as the drug is soluble and can be made up just prior to darting. The high solubility of Zoletil (250 mg/ml) also meant that all animals could be immobilized using a small 1-ml dart, minimizing the risk of physical injury and also the extent of the post darting flight reaction. Zoletil is especially useful for immobilizing wild animals that can be lost before they become recumbent. The very short induction time of this drug (2 min ± 40 s — Table 1) helps to reduce losses of darted animals.

Author's note

Zoletil (CI–744) is not generally available in South Africa and can only be obtained from the distributors under special licence from the Medicines Control Council, Department of National Health, Pretoria. Products containing tiletamine HCl and zolazepam HCl were recently classified as Schedule III drugs by the US Drug Enforcement Administration — *Anim. Pharm.* No 123, 13 February 1987.

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Growth, development and parental investment in the spotted hyaena, *Crocuta crocuta*

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(With 4 figures in the text)

Growth in the spotted hyaena (*Crocuta crocuta*) was investigated using material obtained from the Southern African subregion. A mathematical approximation for post-natal growth of this species was best achieved by a Gompertz equation. An analysis of actual growth in this species revealed that the self-accelerating and self-retarding phases last for up to three years and 10 years, respectively. The exponentially declining growth rate of linear post-natal growth indices suggest that the Huggett & Widdas foetal age estimation procedure may have limited value in this species. Furthermore, some observed limitations of the von Bertalanffy equation for describing post-natal growth in this species are pointed out. The life-history strategy of this reported precocial carnivore is examined in terms of its development and reproductive characteristics.

Contents

Introduction

Despite the abundance of publications dealing with comparative body morphometrics in the spotted hyaena *Crocuta crocuta* (Matthews, 1939; Deane, 1962; Wilson, 1968; Kruuk, 1972; Racey & Skinner, 1979; Whateley, 1980; Hamilton, Tilson & Frank, 1986), very little besides average body dimensions, weights of adult specimens and limited records of birth weights in captivity (Pournelle, 1965; Golding, 1969) are available. The lack of any detailed age-specific morphometric analysis for this species can only be attributed to the absence of an objective method for estimating age. A recently proposed age estimation method based on the occurrence of dentine annuli (van

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Jaarsveld, Henschel & Skinner, 1987) has, however, made the description of spotted hyaena growth feasible.

Extensive predator culling programmes in the Southern African subregion during the 1970s yielded a large quantity of biological material which is unlikely to be repeated. This paper describes the appropriate pre-natal, post-natal and actual growth curves for spotted hyaenas from this subregion. Results indicate that actual growth in this species can be described in terms of a logistic curve. Furthermore, the allometric nature of growth means that, although the Huggett & Widdas (1951) formulation of foetal growth is the only one readily available, it might not be an accurate procedure for this species. Post-natal growth in spotted hyaenas could also not be adequately described using the von Bertalanffy growth equation, and an alternative Gompertz fit was used. Reasons for this phenomenon are suggested and discussed in terms of the life-history strategy of this species.

Materials and methods

Biological material and records used in this analysis were obtained over a number of years (1969–1986) from various sources in Southern Africa.

Foetal material

Spotted hyaena foetuses were collected during culling operations in the Kruger National Park (16) and specifically for this study in the Umfolozi Game Reserve, Natal (2). A total of 18 foetuses were collected from 10 females, 8 of which had twins and the remaining 2 had only 1 foetus each. Foetuses were preserved in 10% formalin or in a mixture of ethanol (96%), formalin (40%), glacial acetic acid and distilled water, in the ratio of 3:1:1:5 (by vol.). Measurements taken were body weight, crown-rump length and vertebral column length, as described by Van Zyl & Skinner (1970). Records of an additional series of 10 foetuses (5 pairs) collected in the Kruger National Park before the start of this project were kindly provided by G. L. Smuts.

Sub-adult material

Body weight, shoulder height, total body length (from the tip of the nose to root of the tail) were measured at irregular intervals from 5 known-age animals (3 litters) born in the National Zoological Gardens, Pretoria. In addition to the captive born litters, sex ratios were collected from 3 litters, in Etosha National Park (2) and Kruger National Park (1).

Adult material

Morphometric data (body weight, shoulder height and total body length) were obtained from 30 hyaenas with age estimated following Van Jaarsveld, Henschel & Skinner (1987). All individuals used in the final analyses emanated from the Kruger National Park, South Africa, population.

Mathematical analyses

Foetal age and specific growth velocity were determined using the formula $a = 3\sqrt{W}/t\text{-}t_0$, where W = foetal weight, a = specific foetal growth velocity, t = gestation age in days, and t_0 = the calculated intercept on the age axis (Huggett & Widdas, 1951). The theoretical value for a was calculated using a birth weight of 1488 g, where $t = 110$ days (Grimpe, 1916; Schneider, 1926) and $t_0 = t \times 0.2$ (see Huggett & Widdas, 1951). Foetal

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TABLE I

Equations fitted to spotted hyaena growth parameters, together with the calculated parameter estimates

Equation	General equation	Growth parameters		
		Shoulder height	Total body length	Body weight
1. von Bertalanffy[1]	$L_t = L[1-e^{-K(t-t_0)}]$ $M_t = M[1-e^{-K(t-t_0)}]^3$	$L_t = 83.69[1-e^{-0.34(t-0.49)}]$	$L_t = 143.5[1-e^{-0.34(t-0.49)}]$	$M_t = 66.85[1-e^{-0.45(t+1.42)}]^3$
2. Gompertz[2]	$y = ab^{r^t}$	$y = (77.49 \times 1.51)^{0.38^t}$	$y = (130.16 \times 1.12)^{0.12^t}$	$y = (66.72 \times 2.16)^{0.60^t}$
3. Logistic[2]	$y = a/(1+br^t)$			$y = 65.76/[1+(9.78 \times 0.44)^t]$

[1] L_t = length in cm at time t; M_t = weight in kg at time t; t = age of animals in years; L and M = asymptote length and weight, respectively; K = coefficient of the rate at which length or weight approach the asymptote; t_0 = a parameter indicating the hypothetical time at which the animal would have been zero size (Smuts *et al.*, 1980).

[2] Asymptotic weight or length = a; b = a function of the potential growth; r = related to the growth rate — if r increases the growth rate decreases and vice versa (Du Toit & Herbst, 1981).

TABLE II

Foetal and post-natal litter sizes, sex ratios and litter compositions of **Crocuta**

	Litter size ($\bar{x} \pm$ S.D.)	Sex ratios ($\male\male:\female\female$)	Litter composition ($\male\male:\male\female:\female\female$)	Sources
Foetal litters	1.80 ± 0.42	$1.00:1.13$	$1:6:3$	This study
n	10	10	10	
Post-natal litters	1.87 ± 0.51	$1.00:0.82$	$2:13:0$	Frank, 1986
n	30	15	15	This study

growth factor (a') was calculated as $a = W_N \cdot G^3$, where W_N = neonatal weight and G = gestation period in days (see Martin & McLarnon, 1985).

Actual growth (pre- and post-natal) in this species was described by fitting a logistic curve to the data and the growth rates (kg/yr) were calculated using a FORTRAN compiler and the 'ST-kromme' computer program (Du Toit & Herbst, 1981), developed in accordance with the mathematical formulations proposed by Steyn, Smit & Du Toit (1981). Post-natal growth was described by estimating the von Bertalanffy equation for the post-natal growth data using a standard iterative, non-linear regression computer program (SAS, 1979, NLIN procedure with MARQUARD option). This procedure was chosen as it: (1) produces the most accurate and precise parameter estimates; (2) sample time intervals need not be equal and; (3) the age class sample sizes need not be identical (Vaughan & Kanciruk, 1982). Post-natal growth was also described by a Gompertz equation (Du Toit & Herbst, 1981) in an attempt to improve the accuracy of post-natal growth description for this species. See Table I for the general forms of these equations and the calculated parameter estimates.
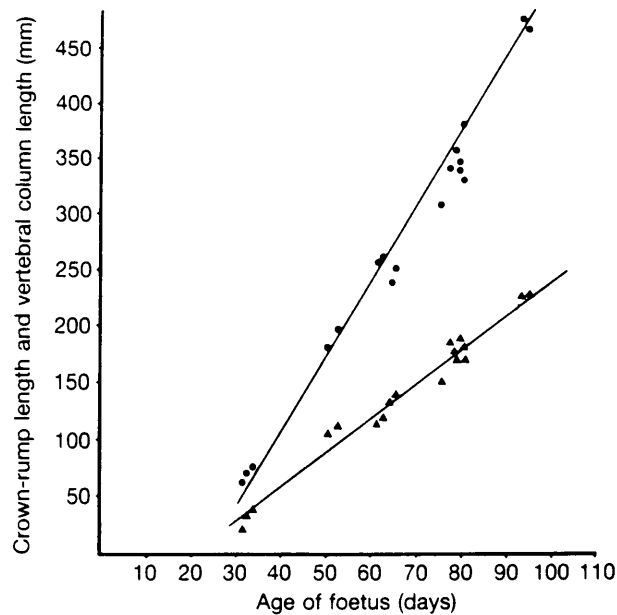
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FIG. 1. The relationship between crown-rump length (▲; $n = 18$) and vertebral column length (●; $n = 18$) of *C. crocuta* foetuses plotted against age, estimated from body weight according to the Huggett & Widdas (1951) procedure.

Results

Sex ratios

The findings of this study, together with published data on sex ratios and litter compositions are summarized in Table II. Mean litter size ($1\cdot87 \pm 0\cdot51$) seems to indicate that twins are the norm for this species. Whereas both foetal and post-natal sex ratios do not differ significantly from the expected 1:1 prediction for mammals ($\chi^2 = 0\cdot4$; $\chi^2 = 0\cdot3$; $d.f. = 1$; Caughley, 1977), there is one interesting aspect that is revealed by the analysis of litter compositions. There has been to date no report of post-natal female siblings in twins.

Pre-natal growth

The state of development at birth for the spotted hyaena is well documented (Pournelle, 1965; Golding, 1969; Kruuk, 1972). Cubs are fully furred at birth, with open eyes, teeth erupted, responsive to sound, and are quite mobile (Pournelle, 1965; Golding, 1969; van Jaarsveld *et al.*, pers. obs.). The calculated specific growth velocity (a) for the spotted hyaena was $0\cdot13$, while the foetal growth factor (a') was $1\cdot12 \times 10^{-3}$. The relationships between crown-rump length and vertebral column length plotted against foetal age, estimated from body weight according to the Huggett & Widdas (1951) procedure, are shown in Fig. 1.
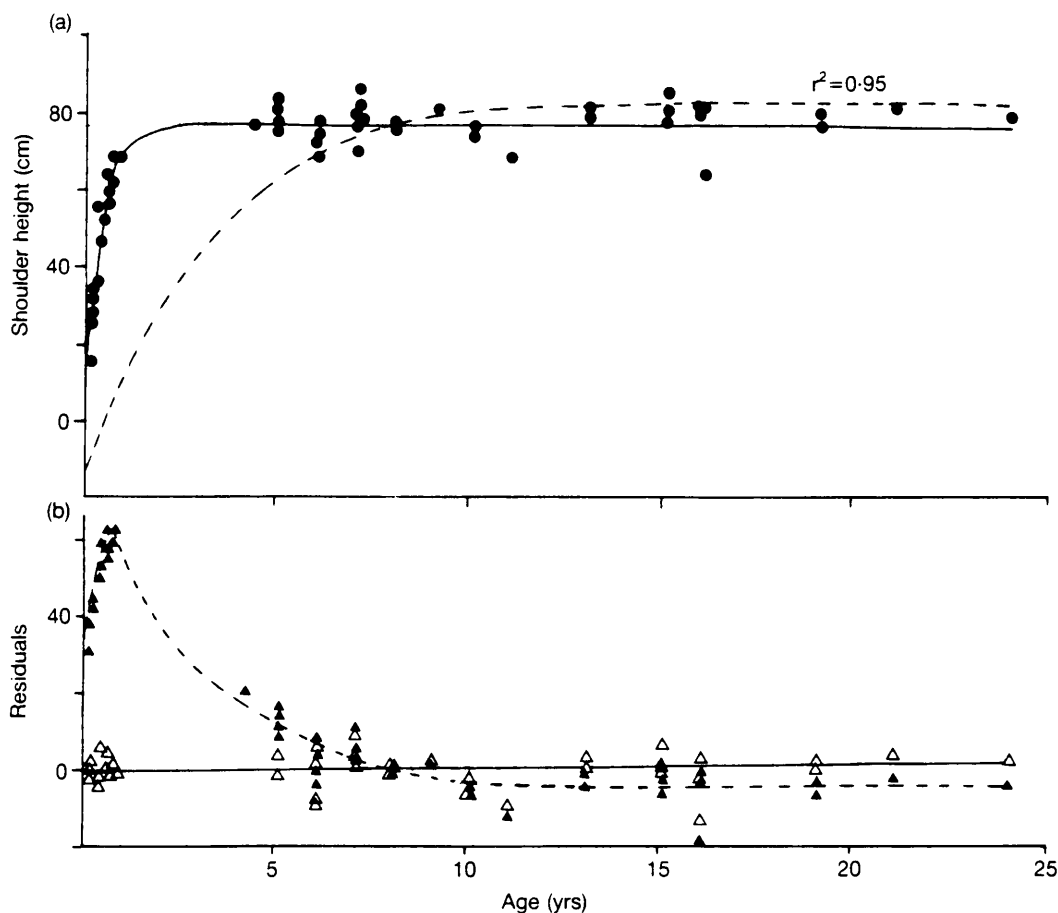
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FIG. 2. (a) Generated Gompertz (——) and von Bertalanffy (- - -) plots for post-natal shoulder height against age in *C. crocuta*. Actual data points are represented by ●; *n* = 51. (b) A plot of calculated residuals for the Gompertz (△, ——) and von Bertalanffy (▲, - - -) equations generated in Fig. 2a, respectively.

Post-natal and actual growth

The obtained curves for post-natal growth, using both von Bertalanffy and Gompertz equations are given in Fig. 2a. The Gompertz fit is significant ($r^2 = 0.95$) and is a more accurate model of post-natal growth for all growth parameters investigated, especially during the early growth phases where the von Bertalanffy equation underestimates the actual growth rate. This trend can be clearly seen in the analysis of residuals for both the von Bertalanffy and Gompertz equations for the variables shoulder height vs. age (Fig. 2b). Similar trends were also observed for age-specific increases in total body length and body weight. Asymptotic weights and lengths derived from these two models, as well as from a logistic model, correspond well (Table I), although the Gompertz model consistently appears more representative of observed post-natal growth.
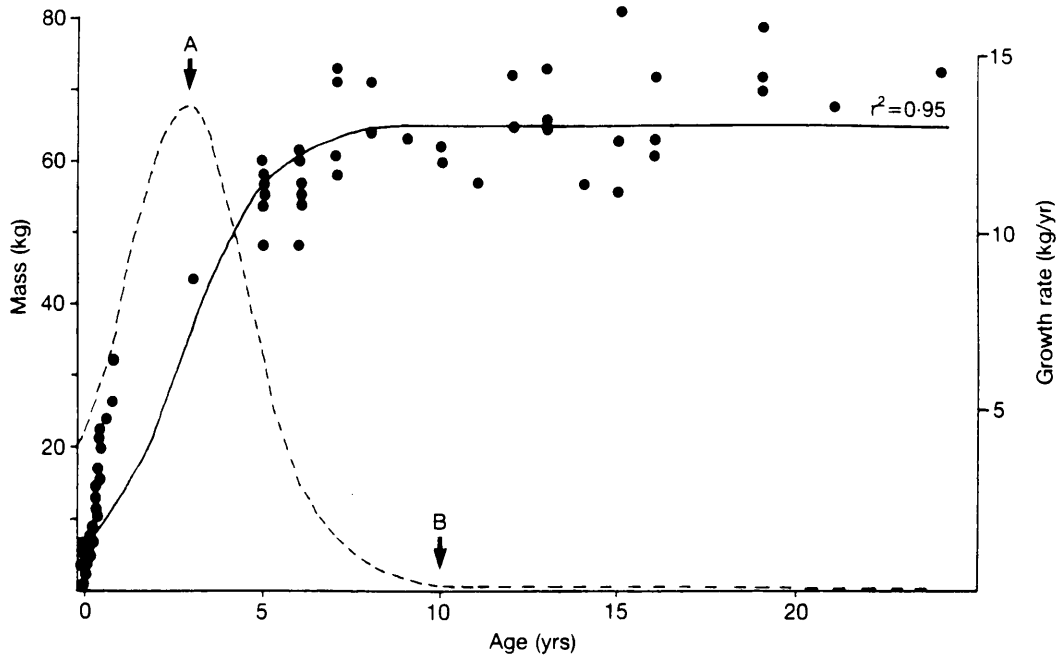
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FIG. 3. A logistic curve (———) generated from actual weight (●, $n = 108$) for *C. crocuta*, together with the calculated growth rates (– – –); A = the inflection point; B = the growth plateau point.

In order to describe actual growth (Batt, 1980) of this species, a logistic curve was fitted to both pre- and post-natal age-specific weight data (Fig. 3). The fit was found to be significant ($r^2 = 0.95$). Therefore, it does seem that growth in this species can be accurately represented through a logistic equation. The three growth phases (Batt, 1980), namely, the self-accelerating phase which lasts for the first three years (inflection point), when the self-retarding phase takes over until 10 years, when the growth plateau is reached (see Fig. 3).

Discussion

Fissiped carnivores are generally believed to be altricial (Case, 1978; Martin & MacLarnon, 1985). The spotted hyaena seems to be the only exception to this general rule, and is to date the only fissiped carnivore reported to be precocial (Kruuk, 1972; Case, 1978). Although these animals can be considered to be morphologically precocial at birth, external morphological factors indicate only in part whether an animal is precocial or altricial. Other characteristics of precocial species include: small litter sizes, and a combination of long gestation periods and low maternal investment levels, relative to maternal mass (Martin & MacLarnon, 1985). In order to understand the life-history strategy of this species it is essential that all these factors be considered.

The foetal growth velocity ($a = 0.13$) of this species does not differ from that expected for most carnivores (range 0.005–0.18; Frazer & Huggett, 1974). The gestation period of 110 days is also similar to that found in felids with similar growth rates (Frazer & Huggett, 1974). Furthermore,
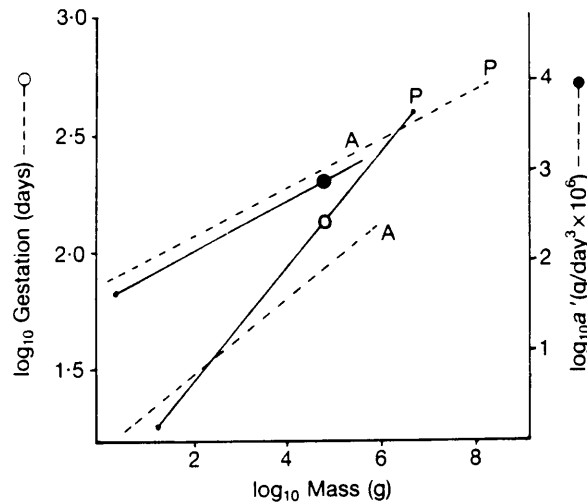
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FIG. 4. Logarithmic plot of (i) gestation period against maternal weight (– – –; ○) and (ii) foetal growth factor (a') against maternal weight (———; ●), for altricial (A) and precocial (P) mammals, respectively, together with the relative position of *Crocuta*, calculated from this study indicated by points ○ and ●. Regression lines were taken from Martin & MacLarnon (1985).

analyses of the calculated foetal growth factor ($a' = 1 \cdot 12 \times 10^{-3}$), litter sizes and gestation period, relative to maternal weight (see Fig. 4) shows that this species falls on the boundary between precocial and altricial mammals, and above the maternal mass point where the separate grades for precocial and altricial species converge (Martin & MacLarnon, 1985). The spotted hyaena can therefore at best be referred to as a semi-precocial mammal, while it can probably be seen as a precocial fissiped carnivore.

Several workers have investigated the comparative morphology of spotted hyaenas. All found that adult females were generally larger than males. This dimorphism has recently been suggested to reflect female access to limited food. Female spotted hyaenas are heavier, though not larger limbed, than males, and this larger body weight is therefore merely a reflection of their dominance as a class over males (Hamilton, Tilson & Frank, 1986). The lack of any skeletal difference in body size (Hamilton *et al.*, 1986; this study) in this species makes the description of sex-specific post-natal growth curves for this species superfluous.

Asymptotic weight (66 kg) is only reached at approximately 10 years in this species (Fig. 3), which indicates a relatively slow post-natal growth rate. Comparably, the lion (*Panthera leo*), which weighs as much as 200 kg, reaches maturity at an earlier age of seven years (Smuts, Robinson, & Whyte, 1980). This relatively slow post-natal growth rate, previously reported by Case (1978), could possibly be attributed to the spotted hyaena's relative precociality compared with other fissiped carnivores, as a lower relative post-natal growth rate is often an indication of an intensive pre-natal maternal investment in individual neonates (Bennett & Harvey, 1985).

There is, however, another characteristic of this species which may play an important role in its life-history strategy, and that is that cubs are fed exclusively on milk for almost the first 9 months of their lives. This must represent an intensive maternal investment in individual neonates during

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the first year. An analysis of residuals for the von Bertalanffy fit of the measured variables vs. age shows that there is a peak in residuals at approximately 9 months of age in all cases (Fig. 2). This means that the inability of the von Bertalanffy equation to approximate the growth data in this species during the early stages of post-natal development is probably a direct result of a fast relative growth rate during the pre-weaning phase, compared to the growth rate following weaning. The residuals in Fig. 2b indicate that the von Bertalanffy model approaches the actual growth rate after weaning ($\pm$9 months). Thus, in this species, it would probably be more realistic to consider the time of weaning as the stage at which the hyaena cubs become independent from the mother, rather than the point of birth as proposed by Huggett & Widdas (1951) for mammals. The inability of von Bertalanffy growth curves to fit the *Crocuta* data can be ascribed to its lack of an exponentially declining growth rate, a property possessed by Gompertz equations (Robinson, McDonald, Fraser & Crofts, 1977).

Although the logistic fit was significant (Fig. 3), one aspect that needs to be considered is that most post-natal growth parameters increase with an exponentially declining growth rate (Gompertz equation) in *Crocuta*. This makes it unlikely that pre-natal growth parameters would follow a different pattern. Furthermore, it is well documented that growth data cannot necessarily be transformed to the linear form (Du Toit & Herbst, 1981) and foetal growth is usually better approximated by a Gompertz curve, which has as an inherent assumption an exponentially declining growth rate (Robinson, McDonald, Fraser & Crofts, 1977; Robinson & McDonald, 1979). This characteristic is generally a feature of foetal growth. There is, however, no method available for estimating foetal age from mass, using a Gompertz fit, as the only biological reference point provided by this equation is an estimate of the time of the inception of Gompertz activity (Robinson & McDonald, 1979). Therefore, if the linear relationship between linear growth parameters (length, height) and foetal age (see Fig. 1), assumed by the Huggett & Widdas (1951) formulation, is incorrect and foetal growth displays Gompertz activity, our foetal age estimation could be incorrect. The use of known-age foetuses could thus significantly improve our model for actual growth in this species.

The fact that both pre- and post-natal sex ratios conform to the expected (1:1) mammalian pattern seems to preclude the existence of any sex-biased reproductive strategy. However, the analysis of litter compositions show that there seems to be a distinct lack of all-female litters among twins. If this pattern is real, the existence of maternal post-natal selection cannot be excluded. It has been suggested that inter-sibling aggression could contribute significantly to cub mortality in this species (Frank, 1986). The ages of the post-natal litters sexed in this study does, however, exclude inter-sibling aggression as a possible mortality factor. Therefore, if some kind of maternal post-natal selection occurs, it is unclear what the selective advantage of such a strategy could be. One possibility is that the raising of two female siblings is detrimental to female philopatry (Henschel & Skinner, 1987), due to the presence of inter-sibling rivalry.

To conclude, our analyses of spotted hyaena growth and development reveals that parameters of post-natal growth are best approximated by an equation which has as an inherent assumption an exponentially declining growth rate (Gompertz equation). This makes the von Bertalanffy equation inappropriate for describing post-natal growth, and possibly, the Huggett & Widdas foetal age estimation procedure inaccurate for this species. Furthermore, these data suggest that the spotted hyaena can at best be referred to as a precocial fissiped carnivore, but should not be seen as a precocial mammal.

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