Endocrine and behavioral changes in male African elephants: Linking hormone changes to sexual state and reproductive tactics

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ABSTRACT

Hormones play a crucial role in mediating genetic and environmental effects into morphological and behavioral phenotypes. In systems with alternative reproductive tactics (ART) shifts between tactics are hypothesized to be under proximate hormonal control. Most studies of the underlying endocrine changes behind ART have focused on fish and amphibians rather than mammals and few have investigated the potential interaction between different endocrine axes in regulating shifts between conditional dependent tactics. Using a combination of endocrine and behavioral data from male African elephants we expand on our previously published analysis and show that the initial increase in androgens predates the behavioral shifts associated with reproductively active periods, supporting the role of androgens in activating sexually active periods in males. A strong interactive effect between androgens and glucocorticoids was found to determine the presence or absence of temporal gland secretion and urine dribbling, signals associated with the competitive reproductive tactic of musth, with elevated glucocorticoids levels suppressing the occurrence of musth signals. In addition external environmental conditions affected hormone levels. The presence of receptive females resulted in elevated androgens in dominant musth males but increased glucocorticoids in subordinate non-musth males. The presented data on hormones, behavior and reproductive tactics strongly support an underlying endocrine mechanism for mediating the translation of intrinsic as well as extrinsic local conditions into the conditional dependent reproductive tactics in male elephants via interactions between the hypothalamic-pituitary-gonadal and -adrenal axes.

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Introduction

The pivotal role of hormones in translating genotypes and environmental effects into morphological and or behavioral phenotypes has attained increasing attention over the last decades (Ketterson and Nolan, 1992; Knapp et al., 2003; Rhen and Crews, 2002). Especially the function of sex steroids in organization or activation of alternative reproductive tactics (ART) has been widely studied and conceptualized in the Relative plasticity Hypothesis (Moore, 1991; Moore et al., 1998). This hypothesis proposes that "plastic phenotypes are switched on and off in the same individual during adulthood by hormonal mechanisms equivalent to activational effects described for sexual differentiation" (Moore et al., 1998). In support of this hypothesis, different androgen levels have been shown to exist between males engaging in different ART in a large number of species including fish (Oliveira et al., 2001; Uglem et al., 2002), reptiles

(Moore et al., 1998), and birds (Wingfield, 1984). Furthermore, partial shifts in tactics can e.g. be obtained in marine iguanas by administering testosterone to individuals (Wikelski et al., 2004) and the probability of non-calling mating tactic in Great Plains Toads can be increased by injection of adrenal corticosteroid (Leary et al., 2006).

ART are widespread in mammals (Isvaran, 2005) but studies of endocrine changes and ART have mainly been conducted on non-mammalian vertebrates. A more general link between androgens, social dominance and reproductive behavior has been shown in mammals (e.g. Pelletier et al., 2003; Creel, 2001) and suggest that shifts between ART in mammals may be controlled by similar mechanisms. The effects of androgens on male reproductive behavior are well documented but the potential role of, and interactions between different endocrine axes in activating ART are less well understood (Knapp, 2004) Additionally, the influence of external social or environmental conditions on ART, potentially via a neuro-endocrinological feedback mechanism, needs further investigation (Knapp, 2004). The hypothalamic-pituitary-gonadal and -adrenal axes are known to interact (Viau, 2002). This has been demonstrated in rats where increasing corticosteroid levels reduce plasma testosterone

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(Monder et al., 1994), and the opposite effect with elevated androgens levels reduce glucocorticoid output in Degus (Kenagy et al., 1999). The latter is consistent with the suppressive effect of testosterone on glucocorticoid in mammals described by Rosner (1990) potentially through a mechanism involving enhanced glucocorticoids feedback regulation (Viau and Meaney, 2004). These findings suggest that interactions between the activity of the hypothalamic-pituitary adrenal (HPA) axis and testicular activity may play a proximate role in determining the onset and termination of reproductive activities or activation of different ART. External social stressors are known to affect glucocorticoid levels in primates (Virgin and Sapolsky, 1997; Wingfield and Sapolsky, 2003; Creel, 2001) hence interactions between androgens and glucocorticoids may especially affect the ART which are influenced by male dominance rank. Ethical and practical considerations largely prevent invasive experiments on larger free ranging mammals. However, a combined analysis of endocrinological changes, temporal timing of changes in behavior, and shifts between ART could be used to assess if hormonal control of behavioral phenotypes are likely in such species and to assess potential interaction between endocrine axes.

Periods of heightened sexual activity and elevated aggression, known as musth, are seen in both Asian (Eisenberg et al., 1971) and African (Poole and Moss, 1981) male elephants. The state of musth is defined on the basis of conspicuous physical signals such as swelling and secretion from the temporal glands (TGS) and urine dribbling (UD) (Poole, 1987). These physical signals are assumed to be honest signals conveying information to competitors of aggressive intent and information to females on high dominance rank (Poole, 1989a). During the 20-year period between puberty around the age of 15 years (Laws et al., 1975; Moss and Poole, 1983) and annual recurrent musth periods around the age of 35 years (Poole, 1987; Rasmussen, 2005) younger non-musth bulls also have distinct sexually active periods where they predominantly associate with female groups (Poole, 1994; Poole et al., 1984; Rasmussen, 2005; Ganswindt et al., 2005b). These younger males obtain matings, have viable sperm (Howard et al., 1984; Hall-Martin, 1987; Barnes, 1982) and sire offspring (Hollister-Smith et al., 2007; Rasmussen et al., 2008) but rank below bulls in musth in the dominance hierarchy (Poole, 1989b). They seldom engage in aggressive interactions and almost never mate-guard receptive females (Poole, 1989b), hence engage in a subordinate, non-competitive reproductive tactic.

Musth is associated with high androgen levels in both African (Ganswindt et al., 2005a; Hall-Martin, 1984; Rasmussen et al., 1996) and Asian elephants (Cooper et al., 1990; Rasmussen and Perrin, 1999; Jainudeen et al., 1972) and behavioral changes like increased walking and reduced foraging (Poole, 1987). Musth bulls are highly aggressive towards other bulls and even non-elephant objects and the majority of escalated fights, occasionally associated with mortality, occur between males where at least one of the contestants are in musth (Poole, 1989a; Hall-Martin, 1987). The aggression and mate guarding behavior is likely linked to the high levels of androgenic steroids known to mediate such behavior in a number of species, (Brain, 1979; Leshner and Moyer, 1975; Ogawa et al., 1996; Rose et al., 1974) including elephants (Ganswindt et al., 2005a). In free ranging African elephants, musth starts to occur around the age of 25–30 years with occurrence and duration positively correlated with age (Poole, 1987) Although musth is positively correlated with age we have previously shown that the elevation in androgen levels in musth bulls is unrelated to age itself (Ganswindt et al., 2005b). In captive bulls where little or no intra sexual competition exist, musth signals have been observed in males as young as 13 years of age (Ganswindt, 2004). Likewise, prolonged periods of musth were observed in young free ranging males (<19 years) during a period where no older males were present but musth was suppressed after the introduction of older bulls (Slotow et al., 2000). Bulls often switch musth signals on and off depending on external conditions starting to signal shortly after encountering an estrous female and ceasing after the arrival of a more

dominant bull in musth (Poole, 1987; Poole, 1989a). This suggests that the sexually active non-musth and musth tactics are conditional dependent tactics (Gross, 1996) and suggest that both testicular and adrenal endocrine axes may be involved in signal regulation and tactic shifts.

In a previous paper (Ganswindt et al., 2005b) we investigated hormonal differences between sexually inactive, active non-musth and musth bulls in a free ranging population of African elephants. We found general differences in epiandrosterone levels between the defined groups with the highest levels found in bulls with both TGS and UD but no overall difference in glucocorticoids. We now expand this initial analysis and, based on a now larger dataset, examine the interactive role of androgens and glucocorticoid in the activation of sexually active periods and shift between ART as well as interactions between local environmental conditions, hormone levels and reproductive tactic. More specifically we investigate i) if an interaction between endocrine axes affect the shift between reproductively inactive and active individuals and between ART, ii) if the relative timing of behavioral and endocrine changes supports an activational role of hormones and iii) if a feed back on endocrine conditions exist from environmental conditions.

Materials and methods

Study area and population

The study area is situated north of the equator on longitude 37°, in and around Samburu and Buffalo Springs National Reserves, Kenya. The area is semiarid savannah with an average precipitation of 380 mm (range 143–847; years 1960–2002). The highly variable annual rainfall occurs during two rainy-seasons in Nov–Jan and April–May.

The elephant population in this area has been monitored by Save the Elephants since 1997 and has been individually identified based on recognizable features such as ear patterns and tusk size and shape (Douglas-Hamilton, 1972; Moss, 1996) The population consists of approximately 220 breeding females, 540 calves and 160 adult males (Wittemyer, 2001; Wittemyer et al., 2005). All adult individuals have been aged based on physical appearance. By using individuals aged from molar progression during immobilizations as reference points these age estimates are within +/−3 years (Rasmussen et al., 2005).

Data collection

Selective monitoring of bulls was conducted continuously between 1999 and 2004. These observations include recording of identity of associating individuals (bulls and females), group composition and the presence or absence of estrous females. The presence and intensity of temporal gland secretion (TGS) and urine dribbling (UD) was further recorded using the methods described by Poole (1987). Between January 2002 and December 2003, 756 fecal samples were collected from fresh droppings for analysis of immunoreactive epiandrosterone (EA) and $3\alpha,11$oxo-cortisol ($3\alpha,11$oxo-CM) concentrations. Samples were obtained from 64 individually identified bulls (median: 11 samples per individual; range 1–68), ranging in estimated age from 18 to 49 years. Of the 756 samples, observations on musth signal presence were incomplete for 26 samples; these samples were excluded from any analysis of signal presence. 13 individuals had a sample frequency allowing for the creation of longitudinal time profiles of at least 1-year duration (median 38 sample/individual; range 15–68) with 9 individuals covering a full 2-year period. In addition, in some individuals with fewer samples, the temporal occurrence of the available hormone samples in respect to the timing of sexually active and inactive periods could be identified (see next section for definition of sexual states). The collection of all samples and observational data was done noninvasively and in accordance with international standards on animal welfare as well as being compliant with both local and national regulations.

Sample classification with respect to sexual state

The initial categorization of the samples as belonging to either sexually active (active bulls) or sexually inactive bulls (inactive bulls) was based on the quantitative shifts in individual bull association patterns known to be linked to shift between reproductive states (Rasmussen, 2005). Periods where individual males were predominantly seen with other males (sexually inactive) or predominantly with females (sexually active periods) was assigned using a probabilistic Hidden Markov Model as described in Rasmussen (2005). Samples belonging to active bulls were further subdivided as belonging to individuals not exhibiting any musth signals (active non-musth) or to individuals exhibiting TGS and UD (musth).

Fecal extraction and hormone assays

The fecal bolus was homogenized by hand (using rubber gloves), and an approximately 10–15 g aliquot was stored in a polyethylene tube, filled with 25 ml of 99% ethanol (Wasser et al., 1988). The samples were stored at 0 degrees at the field site and taken by air in cool boxes to German Primate Centre (DPZ) approx every 2 months. At DPZ the samples were stored at −20 degree's until analysis, which was done soon after arrival. The longest storage time for any sample was approx 3 months hence the samples should not be affected by the potential elevated glucocorticoid measurements following long storage at room temperature reported by Hunt and Wasser (2003).

Fecal samples were extracted and measured for immunoreactive androgen and glucocorticoid metabolite concentrations using validated enzyme immunoassays (EIA) for epiandrosterone (EA) and 3α,11oxo-cortisol metabolites (3α,11oxo-CM) (Ganswindt et al., 2003; Ganswindt et al., 2002) using the procedures described by Ganswindt et al. (2005b). Extraction efficiency, determined in a subset of 20 randomly selected fecal samples by monitoring the recovery of (^{3}H) estradiol added to the samples prior to homogenization, was 72.0% ±5.3% (mean±SD). The epiandrosterone EIA used an antibody raised in a rabbit against 5α-Androstane-3α-ol-17-one-HS, and 5α-Andros-
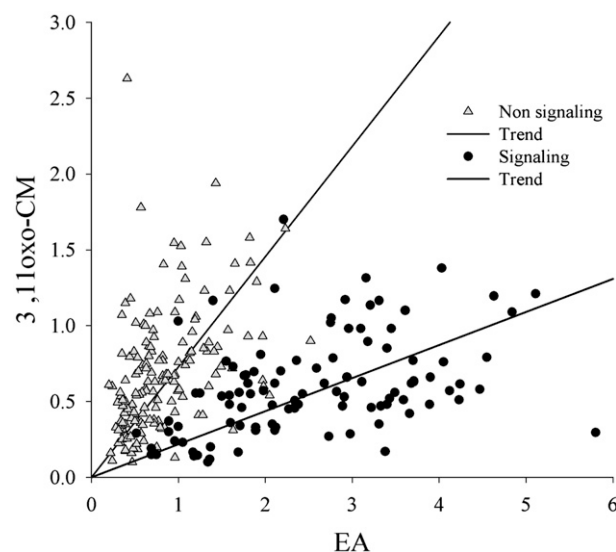

Fig. 1. The combinations of 3α,11oxo-CM and EA levels in sexually active bulls show the negative interaction between the two endocrine axes in determining signals present. Note the heart shaped pattern of hormone combinations (N=286).

tane-3,17-dione-thioether conjugated with biotin as a label (Palm and Möstl, 1993). The 3α,11oxo-CM EIA used an antibody raised in a rabbit against 5β-Androstane-3α-ol-11-one-17-CMO, and 5β-Androstane-3α-ol-11-one-17-CMO conjugated with LC-biotin as a label (Möstl et al., 2002). Sensitivity of the assays at 90% binding were 3.0 pg/well for both EA and 3α,11oxo-CM. Intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low value quality controls ranged between 2.4% and 16.9% for the EA, and 2.6% and 17.5% for the 3α,11oxo-CM measurements.

Due to the food passage time a time delay of approximately 24–36 h exists between actual hormonal changes and until the "signal" can be detected in the feces (Ganswindt et al., 2003). This effect must be considered when comparing hormone levels to specific external events such as antagonistic interaction with competitors or association with females since a sample collected on the day of the event will reflect the hormonal status of the individual the previous day.

Data analysis

The occurrence of sexual activity and TGS+UD in relation to endocrine changes

We used multiple factor logistic regression within a linear mixed model framework (Carlin et al., 2001) to assess the effect of and interactions between the two endocrine axes on male reproductive state (active/inactive) and tactic (non-musth and musth) defined based on presence/absence of TGS and UD. EA and 3α,11oxo-CM concentrations were used as independent continues variables and sexually inactive/active and reproductive tactic (active non-musth/musth) were used as dependent bimodal variables. It has been shown that season affects hormone levels in female African elephants with elevated glucocorticoid levels and reduced levels of progesterone metabolites in feces during the dry seasons (Wittemyer et al., 2007a; Foley et al., 2001). Season was therefore also entered as independent bimodal variable (wet/dry). Season was defined based on mean 10 day NDVI values (Normalized differential Vegetation Index) from the study area. NDVI is a measure of area greenness and is a good indicator of seasonal variation in ecological quality resulting from variation in rainfall (Scanlon et al., 2002). We used previously published NDVI values and season definitions from the study area (Rasmussen et al., 2006) (see also Supplementary material). Because data included multiple measures on the same individual, male identity was incorporated as an effect in the models. The 17 samples

Table 1

Results from multiple logistic regression mixed effect models indicate that the shift from inactive to active non-musth is strongly positively impacted by EA levels but only weakly, negatively impacted by 3α,11oxo-CM levels (note that negative estimate values correspond to positive correlation)

Variables	N (individuals; samples)	Estimate	S.E.	t-ratio full/ reduced	p value	−Log likelihood difference
Model inactive/active	61; 551			227.2	<0.0001	113.5
EA		−1.80	0.20	79.4	<0.0001	
3α,11oxo-CM		1.19	0.23	26.3	<0.0001	
Season		−0.77	0.19	16.5	<0.0001	
Individual ID		−0.0087	0.0024	12.9	0.0003	
Intercept		10.65	2.54	17.6	<0.0001	
Model inactive/non-musth	60; 423			65.6	<0.0001	32.8
EA		−0.87	0.24	13.2	0.0003	
3α,11oxo-CM		0.50	0.24	4.4	0.036	
Season		−0.87	0.20	18.9	<0.0001	
Individual ID		−0.01	0.002	19.9	<0.0001	
Intercept		12.9	2.56	25.5	<0.0001	
Model non-musth/musth	49;256			251.8	<0.0001	125.9
EA		−3.56	0.49	53.8	<0.0001	
3α,11oxo-CM		3.63	0.68	28.30	<0.0001	
Season		0.58	0.51	1.29	0.26	
Individual ID		0.037	0.011	11.33	0.0008	
Intercept		−36.34	11.50	10.04	0.0015	

Contrarily the shift from active non-musth to musth is significantly impacted by both EA level as well as 3α,11oxo-CM level, with increased 3α,11oxo-CM level suppressing musth signals.

belonging to individuals assigned as inactive but exhibiting TGS or UD and the 10 assigned to active individuals but only showing TGS was excluded from this analysis as these samples belonged to the short transition phases between sexual states.

Temporal changes in hormone levels

Individual hormone profiles were examined to evaluate the individual longitudinal variation in hormone concentrations. Profiles of more than 1-year duration were available from 13 individuals and used for this analysis. From these 13 profiles changes in hormone levels between inactive, active non-musth and musth periods were compared using Wilcoxon matched pairs test.

To evaluate the general temporal trends in hormone levels during the course of an active period, results from multiple active periods were combined to average out any individual "noise" potentially linked to specific events. Only results belonging to active periods where the transition between inactive and active could be assessed within 5–10 days were used (i.e. results from individuals where a high temporal resolution of observation existed during the transition

Table 2
Overall hormone concentrations (median and range) during inactive and active periods of musth (TGS and UD) and non-musth bulls

	State	Hormone	N (bulls)	Median µg/g DW	Range µg/g DW
In musth when active	Inactive	EA	10	0.55	0.44–0.62
		3α,11oxo-CM	10	0.58	0.39–0.72
	Active	EA	10	2.41	2.18–3.26
		3α,11oxo-CM	10	0.64	0.47–1.08
Not in musth when active	Inactive	EA	3	0.51	0.44–0.67
		3α,11oxo-CM	3	0.53	0.44–0.55
	Active	EA	3	1.16	0.77–1.20
		3α,11oxo-CM	3	0.88	0.68–0.92

periods). Results from active periods containing a mix of signaling (musth) and none signaling (non-musth) was not included. 289 samples from 23 sexually active periods including the 2-month periods occurring before and after the onset and termination of the active period were selected from 15 individuals. Of those, 205 samples
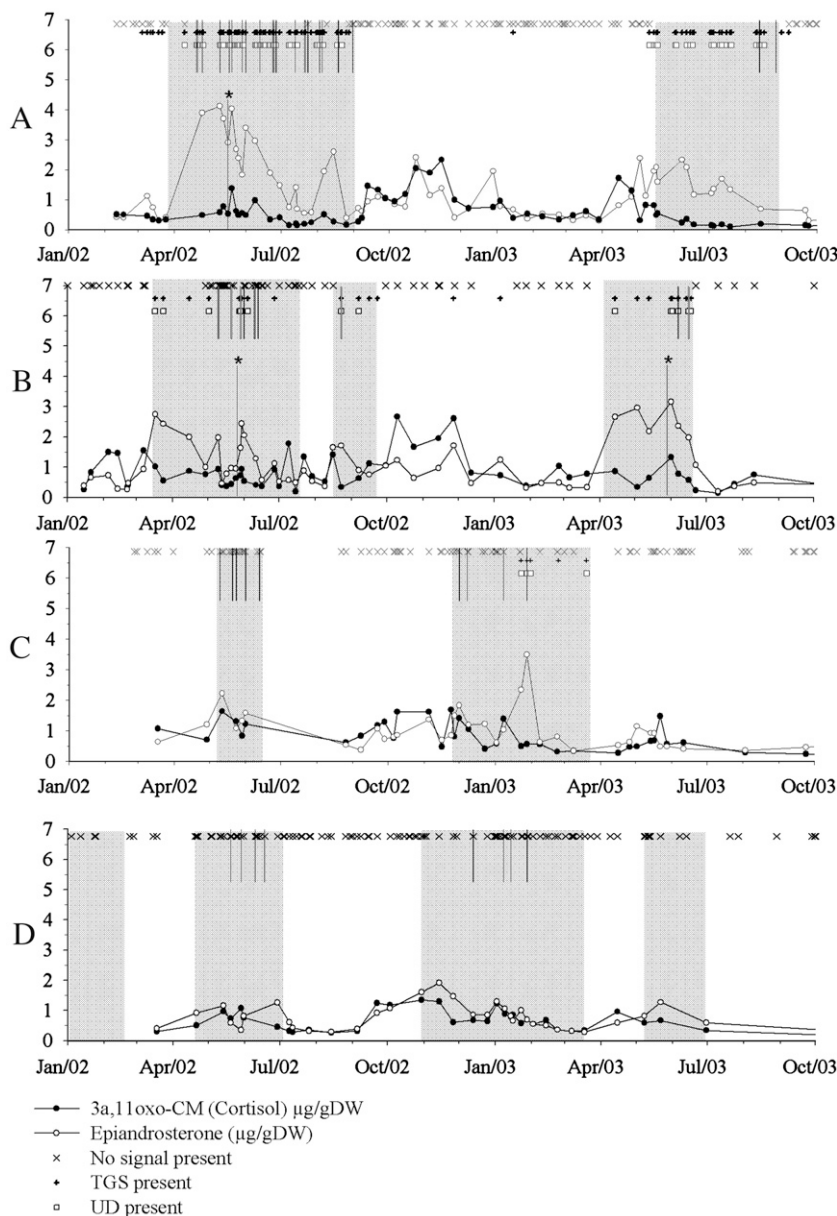

Fig. 2. Longitudinal time profiles of EA and 3α,11oxo-CM concentrations in four bulls of different age (est. age in 2002) (A) 41 years; (B) 37 years; (C) 31 years; and (D) 26 years. Sexually active periods indicated by grey. Top vertical lines indicate observations in association with estrous females. Vertical lines with * indicate fights.

belonged to 9 individuals consistently showing musth signals during the active period. The remaining 85 samples belonged to 6 individuals not in musth.

For musth bulls, samples belonging to the pre and post active periods were grouped into 10-day intervals calculated from the day of period onset and termination respectively. The duration of the active periods varied between individuals (median 98 days, range 53–162). In order to assess any potential changes occurring during the course of an active period the temporal position of samples within active periods were calculated as relative to the duration of the period i.e. as occurring after 20% or 50% of the active period had elapsed. The samples within the active periods were grouped into 10% intervals. In addition all observation of the selected individuals including observations without fecal samples were categorized in a similar way and the fraction of observations with TGS and urine dribbling was obtained. The active periods were defined based on changes in associations. However to assess if the changes in associations occurred abruptly or gradually during onset and termination of the active period, the fraction of observations in bull, solitary or female association were calculated in the same categories.

The sexually active periods of bulls not exhibiting musth signals were analyzed in the same way. However since fewer active periods

with generally fewer fecal samples were available from this group of bulls the categories were enlarged to cover 20-day periods in the pre and post active periods and 20% sections during the active period.

Endocrine changes in relation to environment

The levels of EA and $3\alpha,11$oxo-CM were compared between bulls which had or had not recently been observed associating with an estrous female using Mann–Whitney U-test. Estrus was recorded based on behavioral signs as described in Moss (1983). These behavioral signs have been shown to be highly reliable indicators for ovulation and conception in the study population (Wittemyer et al., 2007b). The 4–6 day estrus period and the up to 72 h delay before the hormonal signal is detected in the feces (Ganswindt et al., 2003) mean that a hormonal signal can be present up to 10 days after the observation with the female. Hence samples from bulls observed in association with an estrous female within the last 10 days prior to sampling were recorded as recently associating. Bulls not observed in association with an estrous female 20 days prior to sampling were recorded as not recently associating. Samples collected between 10 and 20 days following an association with an estrous female were disregarded in this analysis. A total of 90 samples belonged to
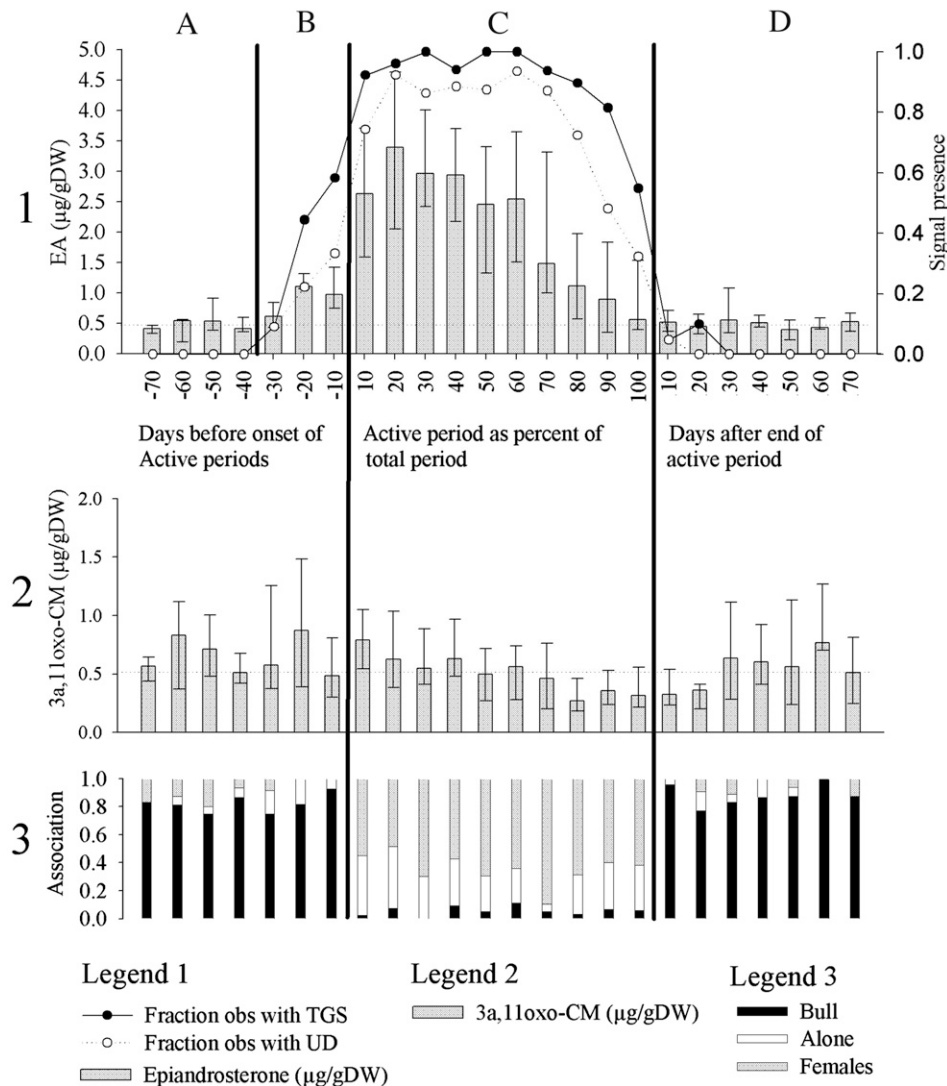

Fig. 3. Median EA level (1) and $3\alpha,11$oxo-CM level (2) ($n=214$ samples) before, during and after a period of sexual activity in signaling bulls ($n=16$ periods from 9 bulls). Fraction of observations with TGS and UD shown under 1 ($n=575$ observations). Time before and after active period is measured inin days whereas time during active period is calculated as percent of total active period to normalizing varying period durations. 3: Fraction of observations in association with bulls, alone or with females (data used for assigning SA periods) ($n=575$ observations).

recently associating bulls (30 bulls; median 1 sample per bull; range 1–12) and were collected during 27 different estrous events (different females; median 1 event per bull; range 1–7) and 149 samples belonged to bulls not recently associating with estrous females (43 bulls; median 2 samples per bull; range 1–14). The effect of encounters with estrous females was analyzed separately within the group of active non-musth and musth bulls. In case of multiple samples from the same individual in the same condition, median values were used for analysis. To evaluate the temporal occurrence of these hormonal changes, the samples for both active non-musth and musth were categorized as belonging to 5 intervals depending on the number of days since they were observed with an estrous female, namely: more than 20 days (not recently associating) 1 day; 2–4 days; 5–10 days and 11–20 days.

Bulls may have been associating with an estrous female without being observed. However the relative low occurrence of estrous females makes it likely that only a small fraction of samples belonging to such bulls were labeled as not recently associating. Likewise, some of the bulls sampled less than 2 days after the association may just have encountered the female on the day of observation so any potential hormonal response would be undetectable in the feces due to the food passage time (Ganswindt et al., 2003). However both types of errors will reduce the power of any analysis making it more difficult to detecting any potential changes in hormone levels.

Results

Sexual active periods and reproductive tactics in relation to endocrine changes

The sexual active state was significantly correlated to both EA and $3\alpha,11oxo\text{-}CM$ levels (Table 1). However the effect of $3\alpha,11oxo\text{-}CM$ levels was small compared to the effect of EA levels when limiting the analysis to sexual active non-musth bulls (Table 1). The shift between reproductive tactics in active bulls, defined based on signal presence was strongly correlated with both EA and $3\alpha,11oxo\text{-}CM$, with signal presence being positively correlated with EA levels but negatively correlated with $3\alpha,11oxo\text{-}CM$ levels (Table 1 and Fig. 1). In addition season had an effect on the onset of active periods. The combination of
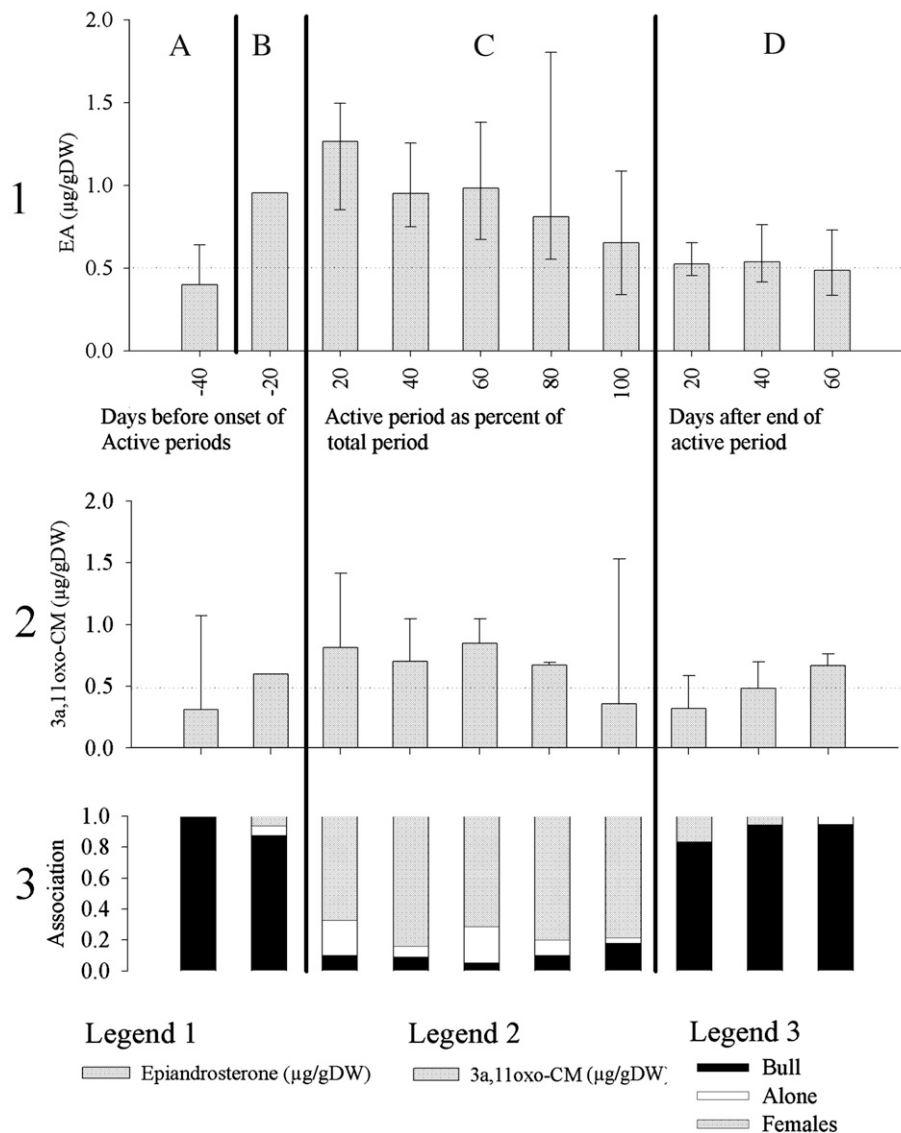

Fig. 4. Median EA level (1) and $3\alpha,11oxo\text{-}CM$ level (2) ($N=84$ samples) before, during and after a period of sexual activity in non-signaling bulls ($N=9$ period in 6 bulls). Time before and after active period is measured in days whereas time during active period is calculated as percent of total active period to normalizing varying period durations. 3: Fraction of observations in association with bulls, alone or with females (data used for assigning SA periods) ($N=315$).

hormone levels had a "heart shaped" appearance (Fig. 1) resulting from the lack of medium/medium or high/high EA and 3α,11oxo-CM combinations (see also Supplementary material Fig. 2).

Individual profiles

Four of the individual profiles are shown in Fig. 2 arranged according to age (see Ganswindt et al., 2005b for 2 additional profiles based on same data set). In the ten bulls predominantly showing musth signals (Figs. 2A and B) no difference in 3α,11oxo-CM levels existed between inactive and musth periods (Wilcoxon matched pair test $z=1.37$; $N=10$; $p=0.17$) (Table 2). Contrary EA were significantly elevated during musth periods compared to inactive periods (Wilcoxon matched pair test $z=2.8$; $N=10$; $p=0.005$) showing a 4–6 fold increase (Table 2). The 10 bulls all showed very similar patterns with the elevated but highly variable EA levels closely linked to the musth period.

In the three bulls with extensive or exclusive active periods without musth signals (active non-musth, Figs. 2C and D) the active periods were associated with 1.5–2 fold increase in 3α,11oxo-CM levels compared to inactive periods. Likewise EA levels were 1.5–2 folds higher during active non-musth periods compared to inactive periods (Table 2). The sample size of only 3 bulls prevented the use of matched pair analysis. However, within each bull both the 3α,11oxo-CM levels and EA levels was significantly elevated during active non-musth periods (Mann–Whitney *U*-test on each individual, EA: $p<0.05$; 3α,11oxo-CM, $p<0.03$). Two of the three bulls (B1026 and B1039) had very short occurrences of musth signals during their active periods (see Fig. 2C for an example). The 4 samples collected during these two incidences (recorded as musth samples and hence not included in the analysis of non-musth levels) were associated with EA levels comparable to bulls exclusively in musth (B1026: 2.34 and 3.49 µg EA/g DW; B1039 3.67 and 2.19 µg EA/g DW).
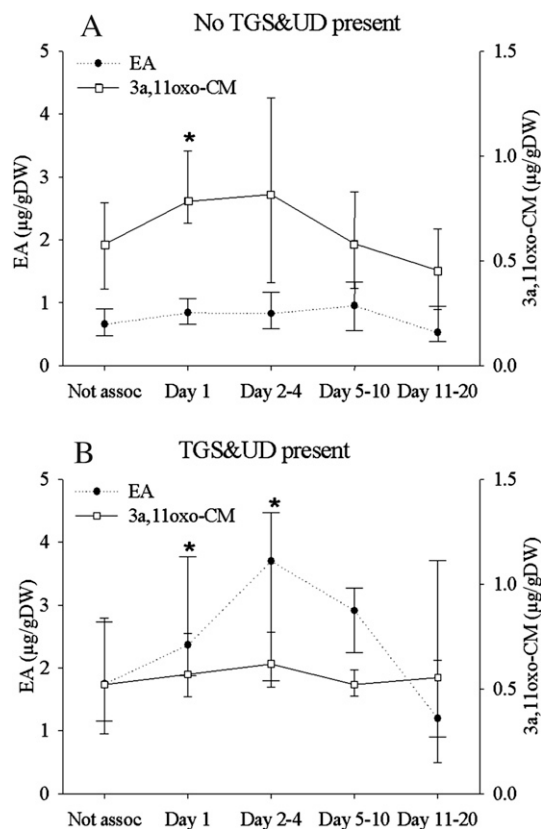

Fig. 5. Temporal changes in EA and 3α,11oxo-CM levels (Median and 25–75 percentiles) following association with an estrous female in non-signaling (A) and signaling bulls (B). Time intervals significantly different ($p<0.05$) from non associating marked with *.

In addition to the reproductively related variation in hormone concentration general elevated levels of both EA and 3α,11oxo-CM was seen in the late dry season 2002. This period of general hormonal increase was seen in all inactive individuals (Supplementary material Fig. 1). Hence this individual based analysis of variation in hormone levels corroborate the results obtained from the multiple factor logistic regression analysis.

Averaged profiles

The averaged profiles of hormone concentrations, signal occurrence and associations of bulls in musth during their active periods are shown in Fig. 3. Up to 30 days prior to the onset of the active period, the EA level was similar to inactive periods and no musth signals were observed (Fig. 3 period A). A month prior to the onset of the active period the EA level started to rise reaching approximately double the level of inactive individuals. Concurrently, musth signals started to appear. At the onset of the active period the EA level rose sharply and peaked during the first 10–20% of the period. At the same time musth signals were seen in over 90% of the observations. After the initial peak the EA level slowly dropped through the course of the active period reaching the level of inactive periods at the point of termination. The musth signals slowly started to disappear during the last 20% of the active period with only a few occurrences of musth signals during the first 10–20 days after termination. The transition in associations, used for defining the active period showed an abrupt shift from 95% bull association to 2% bull association and back at onset and termination of the period rather than a gradual transition (Fig. 3.3). No general trends in 3α,11oxo-CM levels were associated with active periods (Fig. 3.2).

A very similar pattern was seen during active periods of bulls without musth signal. Although only 2 samples were available for the 20 day period prior to the onset of the active period, these samples exhibited an increase in the EA level comparable to that seen in musth bulls (Fig. 4.1 period A). At the onset of the active period the EA level was approximately double compared to when inactive. The peak EA level occurred during the first 20% of the active period and thereafter steadily declined during the active period until the level of inactive periods were reached at the termination. Hence the temporal timing of EA changes in active non-musth bulls was similar to the musth bulls except that the EA level only doubled during the early peak compared to a 6 or 7 fold increase seen in the musth bulls. The maximum level reached by active non-musth bulls during the first part of their active period is thus comparable to the level reached by musth bulls just prior to the onset of their active period (and musth). Like the musth bulls the transition in associations showed an abrupt shift from high to low bull association and back at onset and termination of the period (Fig. 4.3). Contrary to musth bulls, active non-musth bull tended to have slightly elevated levels of 3α,11oxo-CM throughout the active period (Fig. 4.2).

Endocrine changes in relation to environment

In active non-musth bulls, the levels of 3α,11oxo-CM were significantly higher in bulls recently associating with an estrous female whereas EA levels were not different (Mann–Whitney *U*-test: 3α,11oxo-CM: $z=-1.95$; $N=36, 21$; $P=0.05$ EA: $N=36, 21$; $z=-1.10$; $P=0.27$). In musth bulls the opposite occurred with 3α,11oxo-CM levels not significantly different, whereas EA levels increased significantly (Mann–Whitney *U*-test: EA: $z=-2.55$; $N=16, 12$; $P=0.001$ 3α,11oxo-CM: $z=-0.37$; $N=16, 12$; $P=0.71$). The peak in 3α,11oxo-CM levels in active non-musth bulls and EA levels in musth bulls occurred 1–4 days after the bulls were observed with the estrous female (Fig. 5).

The low number of fights observed between males during the study period prevented a proper analysis of the effect of such antagonistic interactions (only 3 fights with associated hormone

samples were available). However short increases in both EA and 3α,11oxo-CM concentrations seemed to be associated with these aggressive encounters including physical interactions (indicated with * in Figs. 2A and B).

Discussion

The combined analysis of the effects of androgens and glucocorticoids on sexual states and musth signals showed that the sexually active non-musth state was positive correlated with immunoreactive epiandrosterone levels (androgens) and weakly negatively affected by glucocorticoid levels (Table 1). For both reproductive tactics, musth and active non-musth, the initial increase in androgens occurred prior to the shift in association preference towards females (Figs. 3 and 4). These results are in accordance with androgens playing a role in activating sexually active periods. Likewise the differences in androgen levels between alternative tactics are in line with the relative plasticity hypothesis. The small increase in androgens above the inactive baseline, seen in active non-musth bulls are probably a breeding baseline needed to trigger reproductive behavior whereas the much larger increase in musth bulls are likely linked to tactics behavior of aggression and mate guarding as conceptualized in the challenge hypothesis (Wingfield et al., 1990). Individual active non-musth bulls showed a significant overall increased in 3α,11oxo-CM levels (glucocorticoids, GC) whereas no increase was seen in musth bulls. This result is consistent with the stress of subordination hypothesis and is contrary to that found in bison (Mooring et al., 2006) a species with a comparable mating system but where dominant individuals experience elevated stress. Dry season negatively affected the onset of sexual activity but not the shifts between tactics. This effect predominately originated from the single atypical long dry season of 2002 with poor ecological conditions occurring after below normal rainfall during 2000 and 2001. During this period above average EA and 3α,11oxo-CM levels was seen in inactive individuals (Table 1 and Supplementary material Fig. 1) and may have been triggered by non-reproductively related environmental factors.

Within the group of sexually active individuals, the presence of TGS and UD, signals indicative of the competitive musth tactic, was strongly linked to both androgen and GC levels. An interactive effect existed between the hormones with signal presence being positively affected by androgens and negatively effected by GC (Table 1 and Fig. 1). This strong interactive effect between the hypothalamic-pituitary-adrenal and -gonadal axes on predicting the signals associated with the musth tactic suggest a combined proximate role of these axes in determining shifts between these alternative tactics. Within active individuals no overall correlation between the two hormones existed although the interaction of the two hormones in determining signal presence resulted in an apparent positive correlation between the two hormones within each group of musth and active non-musth bulls (Fig. 1). This apparent positive correlation between androgens and GC may be counter intuitive to the strong negative effect of GC on signal presence but is caused by the suppressive effect of GC on signal presence. A similar positive correlation between androgens and GC was reported in a study by Brown et al. (2007) and interpreted as a "possible role for the adrenal gland in modulating or facilitating downstream responses" (Brown et al., 2007). Their study was based on captive elephants and they did not identify the state of active non-musth bulls which can only be identified by behavioral signs in free ranging individuals. Hence the reported correlation may have the same origin and in reality result from an underlying negative effect of GC on signal occurrence.

The "heart shaped" pattern of androgen/GC combinations suggest a divergent pattern with either large increase in GC relative to androgens or large increase in androgens relative to GC with the first scenario leading to sexually active non-musth and the later to musth

(Fig. 1 see also Supplementary material Fig. 2) and support the view of musth and non-musth as distinct tactics rather than a continuum. This is suggestive of a negative feedback between the two endocrine axes as previously described in captive elephants (Ganswindt et al., 2005a) However, like Brown et al. (2007) we did not find a direct reduction of GC during musth periods and further studies are needed to clarify the potential feedback mechanism between the HPG and HPA axes.

In musth bulls the association with an estrous female was linked to an increase in androgen but unchanged GC. The opposite occurred in active non-musth bulls which experienced elevated physiological stress but unchanged androgen levels following such events (Figs. 5A and B). The stimulating effect of receptive females on the hypothalamic-pituitary-gonadal axis is known from a number of species and is likely to explain the pattern seen in musth bulls. Musth bulls were present during all estrous events where samples were collected and the physiological stress response seen in non-musth bulls is likely a result of psychological (social) stress resulting in stimulation of the hypothalamic-pituitary-adrenal axis. This link between on one side positive stimulation of the hypothalamic-pituitary-gonadal axis by sexual receptive females and on the other hand stimulation of the hypothalamic-pituitary-adrenal axis from social stressors and the interactions between these axes in determining signal onset and tactic shifts may provide a hormonal mechanism for integrating different stimuli and translating multiple local conditions into alternative conditional dependent behaviors. Bulls can switch urine dribbling on within hours of finding an estrous female and switch it off within minutes of arrival of a much higher ranking musth bull (Rasmussen, 2005; Poole, 1989a) hence showing the rapid response of this signal to changing local conditions. This is in agreement with the interactive effect of the HPA and HPG axes on signal onset and the stimulation of both axes by different external conditions.

Low sample size prevented analysis of the effects of direct antagonistic bull/bull interactions however both androgens and glucocorticoids seemed to have short peaks following such incidences (Figs. 2A, B). The very close association and likely causal link between internal endocrinological conditions and signaling, especially the suppressive effect of GC on musth signals, show that musth signals are indeed honest in the sense that they reflect internal physiological conditions.

Individual musth bulls had highly variable androgen levels during their sexual active period (Fig. 2, see also Ganswindt et al., 2005b). Observations of physical fights or prolonged standoffs with competitors as well as associations with receptive females generally coincided with peaks suggesting external environmental cues were responsible for this pattern. This is in agreement with the challenge hypothesis (Mooring et al., 2006) predicting that the variance in androgens during reproductive active periods is linked to aggression and external stimuli rather than reproductive physiology. On average the highest androgen levels occurred during the first 20% of the active period and may be linked to establishing the dominant position within the group of active bulls during a specific season. The course of inactive non-musth period is somewhat similar except for the lack of the dramatic increase in androgens and the per definition lack of signals.

It is not clear what triggers the initial increase in androgens and hence the shift from sexually inactive to active. A high level of synchrony between years of individual musth periods are seen, with older bulls focusing on only one of two equally good seasons per year but consistently choosing the same. This high level of synchrony is also seen in musth bulls in Amboseli N.P. where some bulls repeatedly have focused on the same time of year for more than 20 years (Poole and Moss, 1981; Poole, 1987; Poole, 1989a). If active periods were simply triggered by internal energy stores the high levels of interseasonal variation in forage availability would be expected to quickly disrupt any cyclic pattern. Likewise general seasonal changes in ecological conditions are unlikely to be responsible as some bulls consistently choose one of two wet seasons, others consistently the

same of two dry seasons. Apparently bulls have an internal annual clock announcing when their time slot has come up.

To conclude, the onset of sexually active periods in male African elephants are likely activated mainly by androgens and the switch between alternative reproductive tactics governed by an interaction between the activity of the hypothalamic-pituitary-adrenal and -gonadal axes. This is in turn directly affected by local external competitive environment and occurrence of receptive females. The increase in androgens following association with estrous females and the link between high androgens and sexual activity and musth signals suggest that estrous events may act as "boosters" prolonging the periods of sexual activity and musth during seasons with many estrous females and contrarily reducing the periods during suboptimal seasons. Hence this would provide an endocrine mechanism for how individual males measure and respond to variation in time specific resource quality. Further studies using higher temporal resolution of observations and samples are needed to reveal if such a mechanism does indeed exist.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yhbeh.2008.05.008.

References

Barnes, R.F.W., 1982. Mate searching behaviour of the elephant bulls in a semi-arid environment. Anim. Behav. 30, 1217–1223.

Brain, P.F., 1979. Effects of the pituitary-gonadal axis on behaviour. In: Brown, K., Cooper, S.J. (Eds.), Chemical Influences on Behaviour. Academic Press, London, pp. 255–329.

Brown, L.B., Somerville, M., Riddle, H.S., Keele, M., Duer, C.K., Freeman, E.W., 2007. Comparative endocrinology of testicular, adrenal and thyroid function in captive Asian and African elephant bulls. Gen. Comp. Endocr. 151, 153–162.

Carlin, J.B., Wolfe, R., Brown, C.H., Gelman, A., 2001. A case study on the choice, interpretation and checking of multilevel models for longitudinal binary outcomes. Biostatistics 2, 397–416.

Cooper, K.A., Harder, J.D., Clawson, D.H., Fredrick, D.L., Lodge, G.A., Peachey, H.C., Spellmire, T.J., Winstel, D.P., 1990. Serum testosterone and musth in captive male African and Asian elephants. Zool. Biol. 9, 297–306.

Creel, S., 2001. Social dominance and stress hormones. TREE 16, 491–497.

Douglas-Hamilton, I. 1972. On the ecology and behaviour of the African elephant.: PhD. Thesis. University of Oxford.

Eisenberg, J.F., McKay, G.M., Jainudeen, M.R., 1971. Reproductive behaviour of the Asiatic elephant (*Elphas maximus maximus* L.). Behaviour 38, 192–225.

Foley, C.A.H., Papageorge, S., Wasser, S.K., 2001. Noninvasive stress and reproductive measures of social and ecological presurres in free-ranging African elephants. Conserv. Biol. 15, 1134–1142.

Ganswindt, A. 2004. Endocrine, physical and behavioural correlates of musth in African Elephants (*Loxodonta africana*). PhD. Thesis, Munster University, Westfalen.

Ganswindt, A., Heistermann, M., Borragan, S., Hodges, J.K., 2002. Assessment of testicular endocrine function in captive African elephants by measurements of urinary and fecal androgens. Zool. Biol. 21, 27–36.

Ganswindt, A., Heistermann, M., Palm, R., Borragan, S., Hodges, J.K., 2003. Non-invasive assesment of adrenal function in the male African elephant (*Loxodonato africana*) and its relation to musth. Gen. Comp. Endocr. 134, 156–166.

Ganswindt, A., Heistermann, M., Hodges, J.K., 2005a. Physical, physiological and behavioural correlates of musth in captive African elephants (*Loxodonta africana*). Physiol. Biochem. Zool. 78, 505–514.

Ganswindt, A., Rasmussen, H.B., Heistermann, M., Keith, J., 2005b. The sexually active states of free-ranging male African elephants(*Loxodonta africana*): defining musth and non-musth using endocrinology, physical signals, and behavior. Horm. Behav. 47, 83–91.

Gross, M.R., 1996. Alternative reproductive strategies and tactics:diversity within sexes. TREE 11, 92–98.

Hall-Martin, A.J., 1984. Plasma testosterone levels in relation to musth in the male African elephant. Koedoe 27, 147–149.

Hall-Martin, A.J., 1987. Role of musth in the reproductive strategy of the African elephants (*Loxodonta africana*). S. Afr. J. Sci. 83, 616–620.

Hollister-Smith, J.A., Poole, J.H., Archie, E.A., Vance, E.A., Georgiadis, N.J., Moss, C.J., Alberts, S.C., 2007. Age, musth and paternity success in wild male African elephants, *Loxodonta africana*. Anim. Behav. 74, 287–296.

Howard, J., Bush, M., de Vos, V., Wildt, D.E., 1984. Electroejaculation, semen characteristics and serum testosterone concentrations of free-ranging African elephants (*Loxodonta africana*). J. Reprod. Fertil. 72, 187–195.

Hunt, K.E., Wasser, S.K., 2003. Effect of long-term preservation methods on fecal glucocorticoid concentrations of grizzly bear and African elephant. Physiol. Biochem. Zoo 76, 918–928.

Isvaran, K., 2005. Variation in male mating behaviour within ungulate populations: patterns and processes. Curr. Sci. India 89, 1192–1199.

Jainudeen, M.R., Katongole, C.B., Short, R.V., 1972. Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elphas maximus*. J. Reprod. Fertil. 29, 99–103.

Kenagy, G.J., Place, N.J., Veloso, C., 1999. Relation of glucocorticosteroids and testosterone to the annual cycle of free-living Degus in semiarid central Chile. Gen. Comp. Endocr 115, 236–243.

Ketterson, E.D., Nolan, V., 1992. Hormones and life histories: an interactive approach. Am Nat 140, 33–62 supplement.

Knapp, R., 2004. Endocrine mediation of vertebrate male alternative reproductive tactics: the next generation of studies. Integr. Comp. Biol. 43, 658–668.

Knapp, R., Hews, D.K., Thompson, C.W., Ray, L.E., Moore, M., 2003. Environmental and endocrine correlates of tactic switching by nonterritorial male tree lizards (*Urosaurus ornatus*). Horm. Behav. 43, 83–92.

Laws, R.M., Parker, I.S.C., Johnstone, R.C.B., 1975. Elephants and their Habitat: the Ecology of Elephants in North Bunyoro, Uganda. Clarendon Press, Oxford.

Leary, C.J., Garcia, A.M., Knapp, R., 2006. Elevated corticosterone levels elicit non-calling mating tactics in male toads independently of changes in circulating androgens. Horm. Behav. 49, 425–432.

Leshner, A.I., Moyer, J.A., 1975. Androgens and agonistic behavior in mice: relevance to aggression and irrelevance to avoidance-of-attack. Physiol. Behav. 15, 695–699.

Monder, C., Sakai, R.R., Miroff, Y., Blanchard, D.C., Blanchard, R.J., 1994. Reciprocal changes in plasma corticosterone and testosterone in stressed male rats maintained in a visible burrow system: evidence for a mediating role of testicular 11beta-hydroxysteroid dehydrogenase. Endocrinology 134, 1193–1198.

Moore, M., 1991. Application of organization–activation theory to alternative reproductive strategies: a review. Horm. Behav. 25, 154–179.

Moore, M., Hews, D.K., Knapp, R., 1998. Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. Am. Zool. 38, 133–151.

Mooring, M.S., Patton, M.L., Lance, V.A., Hall, B.M., Schaad, E.W., Fetter, G.A., Fortin, S.S., McPeak, K.M., 2006. Glucocorticoids of bison bulls in relation to social status. Horm. Behav. 49, 369–375.

Moss, C.J., 1983. Oestrus behaviour and female choice in the African elephant. Behaviour 86, 167–196.

Moss, C.J., 1996. Chapter 7: getting to know a population. In: Kangwana, K.F. (Ed.), Studying elephants. African Wildlife Foundation, Nairobi.

Moss, C.J., Poole, J.H., 1983. Relationships and social structure of the African elephants. In: Hinde, R.A. (Ed.), Primate Social Relationships: An Integrated Approach. Blachwell Scientific, Oxford, pp. 315–325.

Möstl, E., Maggs, J.L., Schrötter, G., Besenfelder, U., Palm, R., 2002. Measurement of cortisol metabolites in faeces of ruminants. Vet. Res. Com. 30, 74–84.

Ogawa, S., Robbins, A., Kumar, N., Pfaff, D.W., Sundaram, K., Bardin, C.W., 1996. Effects of testosterone and 7a-methyl-19-nortestosterone (MENT) on sexual behaviors in two inbred strains of male mice. Horm. Behav. 30, 74–84.

Oliveira, R.F., Canario, A.V.M., Grober, M.S., 2001. Male sexual polymorphism, alternative reproductive tactics, and androgens in combtooth blennies (Pisces:Blenniidae). Horm. Behav. 40, 266–275.

Palm, R., Möstl, E., 1993. Biotin–streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. In: Görög, S. (Ed.), Advances of Steroid Analysis. Akade' miai Kiado', Budapest, pp. 111–117.

Pelletier, F., Bauman, J., Festa-Bianchet, M., 2003. Fecal testosterone in bighorn sheep (*Ovis canadensis*): behaviour and endocrine correlates. Can. J. Zool. 81, 1678–1684.

Poole, J.H., 1987. Rutting behaviour in the African elephants: the phenomenon of musth. Behaviour 102, 283–316.

Poole, J.H., 1989a. Announcing intent: the aggressive state of musth in African elephants. Anim. Behav. 37, 140–152.

Poole, J.H., 1989b. Mate guarding, reproductive success and female choice in African elephants. Anim. Behav. 37, 842–849.

Poole, J.H., 1994. Sex differences in the behaviour of African elephants. In: Short, R., Balaban, E. (Eds.), The Differences Between the Sexes. Cambridge University Press, Cambridge, pp. 331–346.

Poole, J.H., Moss, C.J., 1981. Musth in the African elephant, *Loxodonta africana*. Nature 292, 830–831.

Poole, J.H., Kasman, L.H., Ramsay, E.C., Lasley, B.L., 1984. Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*). J. Reprod. Fertil. 70, 255–260.

Rasmussen, H.B. 2005. Reproductive tactics in male African savannah elephants (*Loxodonta africana*). PhD. Thesis Dept of Zoology Oxford University.

Rasmussen, L.E.L., Perrin, T.E., 1999. Physiological correlates of musth: lipid metabolites and chemical composition of exudates. Physiol. Behav. 67, 539–549.

Rasmussen, L.E.L., Hall-Martin, A.J., Hess, D.L., 1996. Chemical profiles of male African elephants, *Loxodonta africana*: physiological and ecological implications. J. Mammal. 77, 422–439.

Rasmussen, H.B., Wittemyer, G., Douglas-Hamilton, I., 2005. Estimating age of immobilized elephants from teeth impressions using dental silicon. Afr. J. Ecol. 43, 215–219.

Rasmussen, H.B., Wittemyer, G., Douglas-Hamilton, I., 2006. Predicting time specific variation in demographic processes using remote sensing data. J. Appl. Ecol. 43, 366–376.

Rasmussen, H.B., Okello, J., Wittemyer, G., Siegismund, H.R., Arctander, P., Vollrath, F., Douglas-Hamilton, I., 2008. Age and tactic related paternity success in male African elephants. Behav. Ecol. 19, 9–15.

Rhen, T., Crews, D., 2002. Variation in reproductive behaviour within a sex: neural systems and endocrine activation. J. Neuroendocrinol. 14, 517–531.

Rose, R.M., Bernstein, I.S., Gordon, T.P., Catlin, S.F., 1974. Androgens and aggression: a review and recent findings in primates. In: Holloway, R.L. (Ed.), Primate Aggression, Territoriality, and Xenophobia. Academic Press, New York, pp. 275–304.

Rosner, V., 1990. The functions of corticosteriod binding globulin and sex hormone binding globulin: recent advances. Endocr. Rev. 11, 80–91.

Scanlon, T.M., Albertson, J.D., Caylor, K.K., Williams, C.A., 2002. Determining land surface fractional cover from NDVI and rainfall time series for a savanna ecosystem. Remote Sens. Environ. 82, 376–388.

Slotow, R., van Dyk, G., Poole, J., Page, B., Klocke, A., 2000. Older bull elephants control younger males. Nature 408, 425–426.

Uglem, I., Mayer, I., Rosenquist, G., 2002. Variation in plasma steroids and reproductive traits in dimorphic males of corkwing wrasse (*Symphodus melops* L.). Horm. Behav. 41, 396–404.

Viau, V., 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. J. Neuroendocrinol. 14, 506–513.

Viau, V., Meaney, M.J., 2004. Testosterone-dependent variations in plasma and intrapituitary corticosteroid binding globulin and stress hypothalamic-pituitary-adrenal activity in the male rat. J. Endocrinol. 181, 223–231.

Virgin, C.E., Sapolsky, R.M., 1997. Styles of social behaviour and thier endocrine correlates among low-ranking baboons. Am. J. Primatol. 42, 25–39.

Wasser, S.K., Risler, L., Steiner, R.A., 1988. Excretory steroids in primate feces over the menstrual cycle and pregnancy. Biol. Reprod. 39, 862–872.

Wikelski, M., Steiger, S.S., Gall, B., Nelson, K.N., 2004. Sex, drug, and mating role: testosterone-induced phenotype-switching in Galapagos marine iguanas. Behav. Ecol. 16, 260–268.

Wingfield, J.C., 1984. Androgens and mating systems: testosterone-induced polygony in normally monogamous birds. Auk 101, 665–671.

Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am. Nat. 136, 829–846.

Wingfield, J.C., Sapolsky, R.M., 2003. Review article: reproduction and resistance to stress when and how. J. Neuroendocrinol. 15, 711–724.

Wittemyer, G., 2001. The elephant population of Samburu and Buffalo Springs National Reserves, Kenya. Afri J. Ecol. 39, 357–365.

Wittemyer, G., Daballen, D., Rasmussen, H.B., Kahindi, O., Douglas-Hamilton, I., 2005. Demographic status of elephants in the Samburu and Buffalo Springs National Reserves, Kenya. Afr. J. Ecol. 43, 44–47.

Wittemyer, G., Ganswindt, A., Hodges, K., 2007a. The impact of ecological variability on the reproductive endocrinology of wild female African elephants. Horm. Behav. 51, 346–354.

Wittemyer, G., Rasmussen, H.B., Douglas-Hamilton, I., 2007b. Breeding phenology in relation to NDVI variability in free-ranging African elephant. Ecography 30, 42–50.