

The sexually active states of free-ranging male African elephants (*Loxodonta africana*): defining musth and non-musth using endocrinology, physical signals, and behavior

André Ganswindt^{a,*}, Henrik B. Rasmussen^{b,c}, Michael Heistermann^a, J. Keith Hodges^a

^aDepartment of Reproductive Biology, German Primate Centre, 37077 Göttingen, Germany

^bSave the elephants, Nairobi, Kenya

^cDepartment of Zoology, University of Oxford, Oxford OX1 3PS, England

Received 17 March 2004; revised 10 June 2004; accepted 13 September 2004

Available online 22 October 2004

Abstract

Musth in male African elephants, *Loxodonta africana*, is associated with increased aggressive behavior, continuous discharge of urine, copious secretions from the swollen temporal glands, and elevated androgen levels. During musth, bulls actively seek out and are preferred by estrous females although sexual activity is not restricted to the musth condition. The present study combines recently established methods of fecal hormone analysis with long-term observations on male-female associations as well as the presence and intensity of physical signals to provide a more detailed picture about the physical, physiological, and behavioral characteristics of different states of sexual activity in free-ranging African elephants. Based on quantitative shifts in individual bull association patterns, the presence of different physical signals, and significant differences in androgen levels, a total of three potential sub-categories for sexually active bulls could be established. The results demonstrate that elevations in androgen levels are only observed in sexually active animals showing temporal gland secretion and/or urine dribbling, but are not related to the age of the individual. Further, none of the sexually active states showed elevated glucocorticoid output indicating that musth does not represent an HPA-mediated stress condition. On the basis of these results, we suggest that the term “musth” should be exclusively used for the competitive state in sexually active male elephants and that the presence of urine dribbling should be the physical signal used for defining this state.

© 2004 Elsevier Inc. All rights reserved.

Keywords: African elephant; *Loxodonta africana*; Sexual activity; Musth; Reproductive strategy; Stress; Androgens; Glucocorticoids; Feces; Non-invasive methodologies

Introduction

Male African elephants leave their natal families at the onset of puberty around 12–15 years of age (Poole, 1989b, 1994). From this point in time, they live in a highly dynamic world of changing sexual state, rank, associations, and behavior. Most of the time they live alone or in small groups with other males, except during sexually active

periods when they predominantly associate with females (Poole, 1987, 1989b). During sexually active periods, individuals sometimes enter into a state known as musth (Hall-Martin, 1987; Poole, 1987, 1994; Poole and Moss, 1981), a condition initially recognized in adult Asian bulls in captivity (e.g., Eggeling, 1901), but now also known to occur in the African species (Poole and Moss, 1981). Musth in African elephants was originally defined by Poole, based on the presence of a combination of two main physical signals; secretion from the temporal glands (TGS) and urine dribbling (UD) (Poole, 1982, 1987). In addition to TGS and UD, a sharp rise in aggressive behavior as well as a number of more subtle changes in displays and posture

* Corresponding author. Department of Reproductive Biology, German Primate Centre, Kellnerweg 4, 37077 Göttingen, Germany. Fax: +49 551 3851288.

E-mail address: ganswindt@dpz.gwdg.de (A. Ganswindt).

(see Kahl and Armstrong, 2002) have also been assigned to musth. Musth is also known to be associated with restlessness and reduced feeding activities (Poole, 1982, 1989a) and physiologically, with elevated androgen levels (Hall-Martin, 1987; Poole et al., 1984; Rasmussen et al., 1996) and an additional definition of musth based on the occurrence of a threshold level of testosterone in serum has been proposed (Rasmussen et al., 1996).

The occurrence and duration of musth periods (defined by TGS and UD) are positively correlated with age (Poole, 1987, 1989b). This, combined with the increase in aggression towards competitors and elevated androgen levels, suggests that musth is a special age-related form of sexual activity in which the individual is in a highly competitive mode. The existence of such age related changes in reproductive strategy in elephants would, in fact, make sense, since in a system in which post-mature growth and size-dependent competition for mates exist, a competitive strategy like musth would not be expected to pay before late in life (Whitehead, 1994). The age-relatedness of the signals used to define musth, however, raises the possibility that other, behavioral and/or endocrine changes commonly associated with these signals, may in part be related to the age of the sexually active individual rather than exclusively to the occurrence of musth itself. Indeed, in a study by Poole et al. (1984), high levels of androgens, comparable to those showed by animals in musth, were found in some of the sampled individuals assumed to be sexually active but not showing musth signals. Thus, within the context of sexual activity, “full” musth and sexually active non-musth could represent end points in a continuum with a graduate onset of signal presence and intensity linked to age, rather than being distinct states. A combined analysis of the degree and occurrence of the various physical signals in relation to age, physiology, and behavior is needed in order to understand the link between these various factors. Although it is widely accepted that musth plays an important part within the reproductive context (Kahl and Armstrong, 2002; Rasmussen and Schulte, 1998), its occurrence is not an explicit requirement for male mating ability, since non-musth bulls are known to mate successfully and have comparable sperm quality (Hall-Martin, 1987; Poole, 1989b; Rasmussen and Schulte, 1998). Thus, at least two different reproductive strategies appear to exist in African elephants, namely musth and sexually active non-musth. To date however, the majority of research on male African elephant reproduction has concentrated (almost exclusively) on the state of musth and limited information is available on the characteristics of the sexually active non-musth state. An unquantified increase in degree of association with females in the absence of musth signs is the only manifestation for the sexually active non-musth condition described so far (Poole, 1987; Poole et al., 1984), making it difficult to clearly distinguish between sexually active non-musth and sexually inactive individuals.

Although Poole et al. (1984) have reported on androgen levels in sexually active non-musth bulls, data are limited (five individuals, single samples) and values obtained showed the full range from those in sexually inactive bulls to those in musth. Thus, in terms of androgen levels, it remains unclear if and how sexually active non-musth bulls can be differentiated from individuals in musth. Additionally, it would be interesting to know if animals showing TGS only differ in their androgen concentrations from those showing UD. Another point of interest, which remains to be addressed in free-ranging animals, is whether either of the reproductively active states is associated with increased adrenal activity. For female African elephants, it has been shown that limited access to food and water, which leads to a decline in body condition, is associated with an increase in adrenal endocrine function resulting in elevated glucocorticoid levels (Foley et al., 2001). Since the competitive condition of musth is known to be associated with increased restlessness and reduced feeding activities, which often leads to a significant weight reduction and progressive loss of condition (Barnes, 1982; Poole, 1982, 1989a), it would be reasonable to assume that musth may be associated with an elevation in glucocorticoid output.

In this paper, we use a combination of quantified association preferences, age, as well as the presence and intensity of physical signals to establish reliable categories to provide a more detailed picture about the physical and behavioral characteristics of the different states occurring within periods of sexual activity. Further, we examine if the different existing reproductive conditions are associated with quantitative differences in androgen excretion and finally, whether states of sexual activity represent a form of physiological stress for free-ranging male African elephants.

Materials and methods

Study area and population

The study area is situated in and around the Samburu and Buffalo Springs National Reserves, Kenya, approximately 80 km north of the equator on longitude 37° East. It covers approximately 10,000 km² of which less than 10% are within national parks or reserves. The area consists of low-lying semi-arid rangeland along the Ewaso Ngiro River, the only semi-permanent river in the area. The region is dry and hot throughout most of the year with the average 350 mm of rain mainly falling during April/May and November/December. The total elephant population within the Samburu/Laikipia ecosystem is estimated to number around 5000 individuals and is the largest free ranging elephant population in Kenya occurring mainly outside protected areas (Omondi et al., 2002). Approximately 900 individuals, including 150 adult bulls, occur within the core study area of Samburu and Buffalo Springs National Reserves and have

been individually identified by the Save the Elephants ongoing monitoring program (Wittemyer, 2001).

Observations and sample collection

In addition to the general monitoring program, a selective monitoring of bulls has been continuously performed within the study area since 1999. During these observations, the identity of all associating individuals (males and females) was recorded. Furthermore, for each bull the presence and degree of urine dribbling (UD), temporal gland swelling (TG) and temporal gland secretion (TGS) (all common visible signs assigned to musth) was rated on an increasing scale as described by Poole (1987).

In addition, between January 2002 and May 2003, 660 fecal samples were collected for hormone analysis from 64 individual bulls (mean: 15 samples per individual, range: 3–56), ranging in age from 18 to 49 years. The bulls were aged by physical appearance (facial shape, tusk size, and general appearance) (Moss, 1996; Poole, 1987) using 15 bulls accurately aged by molar progression (Jachmann, 1985; 1988; Laws, 1966) during immobilization as reference points, giving an accuracy of less than ± 3 years (Rasmussen et al., submitted publication). Samples were collected from fresh droppings of identified individuals. 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 100% ethanol (Wasser et al., 1988). The samples were refrigerated until transport to the German Primate Centre for analysis.

Fecal extraction and hormone assays

Fecal samples were homogenized in the original ethanolic solvent according to the procedure described by Ziegler et al. (2000). The ethanolic fecal suspension of each sample (including a 3×5 ml 80% methanolic rinse of the original sample tube) was then transferred into two 50-ml tubes, and steroids were extracted by shaking for 15 min on a multi-tube vortex. Following centrifugation at 3000 rpm for 15 min, both supernatants were combined, the total volume recorded, and the extracts finally stored at 4°C until hormone analysis. The remaining fecal pellets were dried in a vacuum oven at 50°C, and the dry weight of individual samples was determined. Extraction efficiency, determined in a subset of 20 randomly selected fecal samples by monitoring the recovery of (³H)estradiol added to the samples prior to homogenization, was $72.0\% \pm 5.3\%$ (mean \pm SD).

Fecal extracts were measured for immunoreactive epian drosterone (EA), a major secretory steroid of the mature boar testes (Raeside et al., 1992), which have been shown to be potentially useful for monitoring male gonadal function in different mammalian species (e.g., Möhle et al., 2002; Palme and Möstl, 1993) including African elephants (Ganswindt et al., 2002). In addition, in all fecal samples

collected, glucocorticoid concentrations were measured using a validated enzyme immunoassay (EIA) for 3 α ,11oxo-cortisol metabolites (3 α ,11oxo-CM) which have previously been shown to provide reliable information on adrenal function in the male African elephant (Ganswindt et al., 2003). The epian drosterone EIA used an antibody raised in a rabbit against 5 α -androstane-3 α -ol-17-one-HS and 5 α -androstane-3,17-dione-thioether conjugated with biotin as a label (Palme and Möstl, 1993). The 3 α ,11oxo-CM EIA used an antibody raised in a rabbit against 5 β -androstane-3 α -ol-11-one-17-CMO and 5 β -androstane-3 α -ol-11-one-17-CMO conjugated with LC-biotin as a label (Möstl et al., 2002). Cross-reactivities of the two antibodies are described in Ganswindt et al. (2002) for EA and Möstl et al. (2002) and Huber et al. (2003) for 3 α ,11oxo-CM. Serial dilutions of fecal extracts gave displacement curves which were parallel to the respective standard curve in both assays. Sensitivity of the assays at 90% binding was 3.0 pg/well for both EA and 3 α ,11oxo-CM. Intra- and interassay 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 3 α ,11oxo-CM measurements.

Sample classification in relation to sexual behavior

In order to avoid an a priori categorisation based on signal levels, the initial categorisation of the samples as belonging to either sexually active (SA) or sexually inactive (SI) bulls was solely based on quantitative shifts in individual bull association patterns. Each observation of an individual bull (including observations without collection of fecal samples) was categorized as belonging to one of three group types and given values from 1 to 3. The group types were: (1) in association with other bulls only; (2) alone, no signs of association with other individuals; and (3) in association with mature females, regardless of the presence of other bulls. Individuals were regarded as being associating if they were in close proximity and moving in a coordinate way at the time of observation (individuals are often clustered within a relative small area, thus making associations easily recognized).

In order to exclude the probability that the type of association seen on the day of sample collection was not representative, an average association index was calculated by averaging the group type of the previous, present, and following observation. If an observation was separated from other observations by more than 2 weeks, the association index was equaled to the observation group type. The bulls showed clear patterns of shifts in the association index over time (see Fig. 1). Since bulls are known to leave their retirement areas and search out and associate with females only during definite periods of sexual activity (Moss and Poole, 1983), periods with an index value of >2 (mainly in association with females) were considered to represent periods in which the bull was sexually active (SA), whereas

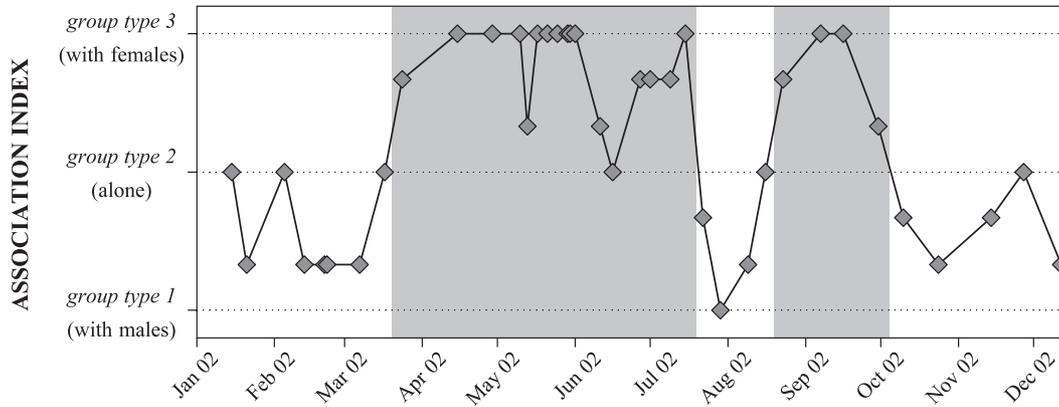


Fig. 1. Profile based on the pattern of bull association with females for the adult male Apollo-B1033 (est. born 1965) throughout year 2002. Grey bars indicate the periods in which the bull was assigned to be sexually active.

these with an index value of <2 (mainly with other bulls) were considered to represent a sexually inactive (SI) state. During transition phases between periods of SA and SI, the observation of specific group types was investigated to pinpoint the date of transition.

Samples coming from SA bulls were further subdivided depending on the degree and presence of TGS, TG, and UD, initially scored according to the definitions described by Poole (1987). However, this resulted in too many different categories (>100), reducing the sample size in each to below a reasonable level. All samples were therefore re-assigned and the occurrence of TG was considered as an automatic effect of changes in TGS and not considered for sample subgrouping. Samples were then assigned if TGS was absent (–), present low (1), medium (2), or high (3) (as described by Poole, 1987) in combination with the absence (–) or presence (+) of UD, giving a total of 8 potential sub-categories for sexually active bulls (SA) plus one category for sexual inactivity (SI). Since urine dribbling at no point was observed without any temporal gland secretion, the final number of categories was 7 SA + SI (see Table 1).

Data analysis

When multiple samples from the same individual in the same category existed, the median value was used for

analysis. Differences in hormone concentrations between conditions (see Tables 1 and 2) were examined by Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks followed by all pairwise post hoc analysis using Mann–Whitney Rank Sum Test. All tests were two-tailed, with the α -level of significance set at 0.05. In cases of all pairwise multiple comparison procedures, the α -level was adjusted by applying the procedure described by Holm (1979). Non-parametric Spearman Rank Order or Pearson Product Moment correlation coefficients were calculated for the relationships between median hormone levels within separate conditions (see Table 1) and age of the animal. The computer program Jandel Sigma Stat® version 2.0 was used for all statistical analyses.

Results

During the 17-month study period, 73% ($n = 47$) of all bulls were observed to be sexually active on at least one occasion and of those, 83% ($n = 39$) were at least partially observed in a sexually active condition without showing any TGS or UD (SA[–]). Moreover, 49% ($n = 23$) of all SA bulls were observed with an additional occurrence of TGS (SA[–]), and 40% ($n = 19$) showed both, TGS and UD (SA⁺⁺).

Table 1

Categories of study animals according to the state of sexual activity, presence, and degree of temporal gland secretion (TGS) and urine dribbling (UD) and age

Category	Presence of SA	Presence of UD	Amount of TGS	Abbreviation	No. of individuals	Age (years)	
						Median	Range
1	–	–	–	SI	52	28	18–49
2	+	–	–	SA [–]	39	24	18–44
3	+	–	1	SA ^{–1}	9	37	18–44
4	+	–	2	SA ^{–2}	4	38	18–41
5	+	–	3	SA ^{–3}	6	39	24–48
6	+	+	1	SA ⁺¹	6	35	25–48
7	+	+	2	SA ⁺²	9	39	27–48
8	+	+	3	SA ⁺³	15	38	25–48

Table 2

Finally established categories with respect to the state of sexual activity and the presence/absence of temporal gland secretion (TGS) and urine dribbling (UD)

Category	Presence of SA	Presence of UD	Presence of TGS	Abbreviation	No. of individuals
1	–	–	–	SI	52
2	+	–	–	SA ⁻⁻	39
3	+	–	+	SA ⁻⁺	13
4	+	+	+	SA ⁺⁺	19

Individual androgen and glucocorticoid excretion

Fig. 2 shows the duration and occurrence of sexual activity, temporal gland secretion and urine dribbling in combination with profiles of fecal EA and $3\alpha,11\text{oxo-CM}$ immunoreactivity over the whole study period in two adult males, Mungu (B1001) and Esidai (B1027). The 48-year-old bull Mungu was observed to be sexually active on two occasions during the study, whereby the first period lasted 11 weeks and the second period started with the last sample point at the end of the study period. Each of the SA periods of B1001 was associated with the occurrence of both TGS and UD and a clear elevation in EA (up to 10-fold). The 38-year-old bull Esidai showed three periods of sexual activity (2 complete, 1 incomplete), all of different length (3, 5, and 14 weeks). SA periods where TGS and/or UD occurred were associated with clearly elevated (5- to 10-fold) levels of EA, but no clear increase was observed during SA periods without signals. Apart from the SA-associated elevations in

EA, an additional 2-month period of moderately elevated levels of EA was found in both animals during the end of the long dry season 2002 (mid September to mid November). In contrast to the course of EA, no elevation of $3\alpha,11\text{oxo-CM}$ levels during the SA periods of either animal can be seen. Interestingly, there was a small but clear elevation in $3\alpha,11\text{oxo-CM}$ levels in both bulls during the end of the long dry season in 2002 (Sept.–Nov.) coinciding with the period of non-sexual related elevation in EA.

Endocrinological changes in relation to age

The age distributions of bulls within each of the 7 subcategories of SA were significantly different (One Way Analysis of Variance; $F_6 = 6.562$, $P < 0.001$); in particular, the median age of bulls showing TGS and/or UD was higher than that of SA individuals showing no additional signs (Table 1). The non-parametric nature of the data prevented an overall full factorial analysis for age and signal effects.

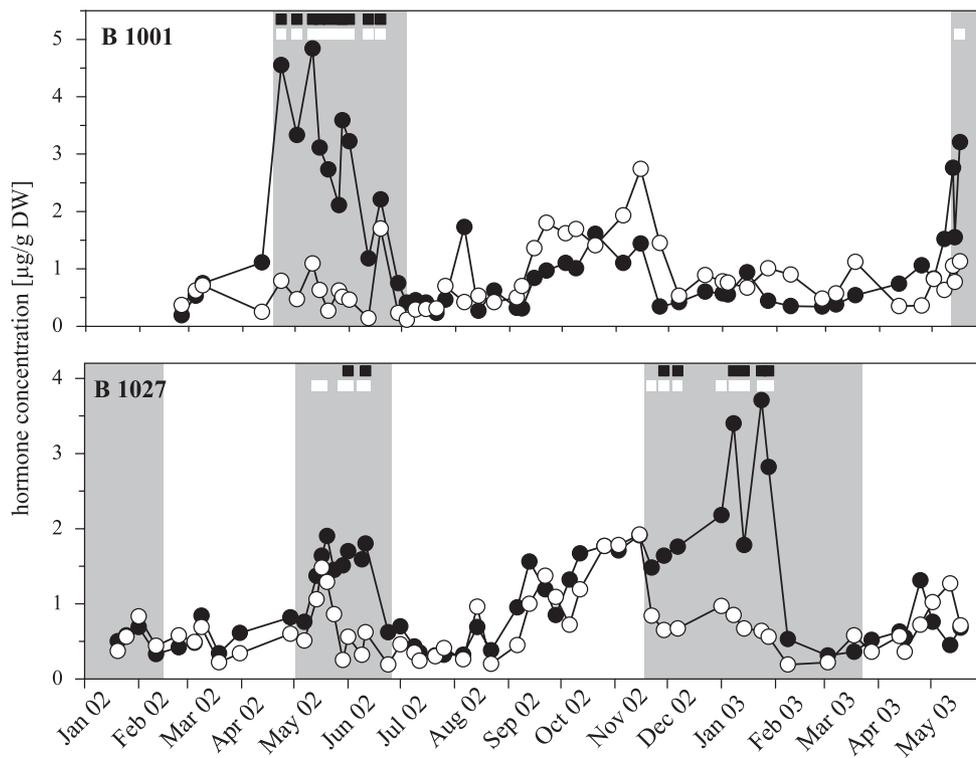


Fig. 2. Profiles of fecal EA (●) and $3\alpha,11\text{oxo-CM}$ (○) immunoreactivity throughout a 17-month period in two adult male African elephants (B1001 and B1027). Grey bars indicate the periods of sexual activity. Boxes indicate the periods during which signs of TGS (white) and UD (black) were recorded.

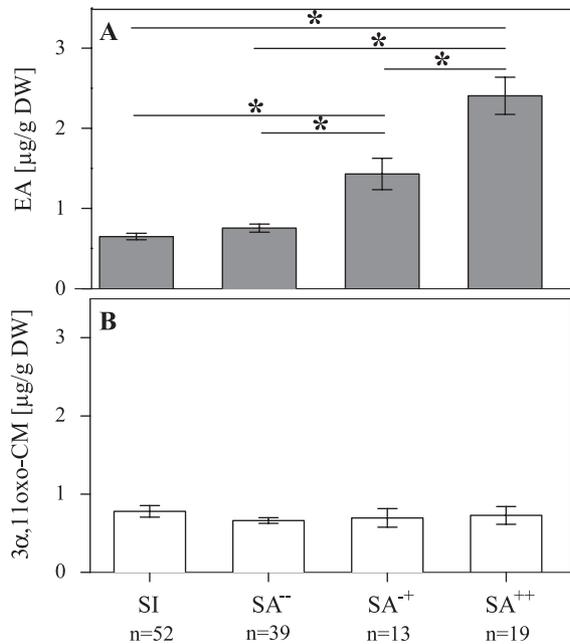


Fig. 3. Vertical bar plots of grouped concentrations of EA (A) and 3 α ,11oxo-CM (B) representing data derived from samples of 64 bulls. The bars represent the overall mean hormone value \pm standard error of samples collected from individual bulls in different conditions of sexual activity. Group SI includes all sexually inactive individuals. The SA⁻ subgroup includes all bulls which showed sexual activity but no UD and TGS. The both other subgroups includes all SA bulls which showed additionally some physical signs and were divided in SA⁺ and SA⁺⁺ based on the absence/presence of UD (see Table 2). The asterisks indicate statistically significant difference between categories.

Each of the seven subgroups were therefore analyzed separately for age effects on EA and 3 α ,11oxo-CM levels (using Spearman Rank Order or Pearson Product Moment correlation depending on normal distribution of the data). Neither EA nor 3 α ,11oxo-CM levels were significantly correlated with age in any of the 7 SA subgroups even before α -level adjustment ($R_{3-38} = -0.91$ to 0.55 ; $P = 0.878-0.078$). Age was therefore excluded as a factor in subsequent analyses.

Endocrinological changes in relation to the degree of TGS

The effect of increasing levels of TGS on androgen and glucocorticoid levels was investigated in the subgroups with (SA⁺¹, SA⁺², SA⁺³) and without (SA⁻¹, SA⁻², SA⁻³) urine dribbling. The variance of the overall median hormone values (EA and 3 α ,11oxo-CM, respectively) within each of the three respective conditions was relatively low and no significant differences could be found between the respective categories (Kruskal–Wallis one-way ANOVA on ranks, $H_2 = 5.098-0.301$, $P = 0.860-0.078$).

Since the intensity of TGS had no relationship with hormone levels, the samples were assigned according to whether TGS was absent (–) or present (+) in combination with the absence (–) or presence (+) of UD, thus giving a total of three potential sub-categories for sexually active bulls

(since UD does not occur without TGS), with one additional category for sexually inactive animals (SI) (see Table 2).

Endocrinological changes in relation to different forms of sexual activity

In Fig. 3, EA and 3 α ,11oxo-CM levels representing samples from all 64 bulls categorized according to the four established groups (1 inactive and 3 active) are shown. Within the group of sexually inactive bulls (SI) and sexually active animals showing no physical signs (SA⁻), the variance in the individual androgen levels was relatively low (SI: 0.60 ± 0.30 μ g/g, SA⁻: 0.69 ± 0.34 μ g/g), compared to both the SA⁺ (1.40 ± 0.67 μ g/g) and SA⁺⁺ subgroups (2.35 ± 1.01 μ g/g). Median EA levels of all groups were different from each other except SI and SA⁻ individuals (Kruskal–Wallis one-way ANOVA on ranks, $H_3 = 57.363$, $P < 0.001$, post hoc analysis using Mann–Whitney Rank Sum Test, α -level adjusted to 0.008). In contrast to the data for EA, no significant differences were found in 3 α ,11oxo-CM levels between the four categories (Kruskal–Wallis one-way ANOVA on ranks; $H_3 = 0.567$, $P = 0.904$). To verify that the non-significant effect of age on EA levels found in the initial eight subcategories was not a result of low sample size, age effects were re-analyzed within the final four groups. Again, no significant effects of age were found in any of the groups (Spearman Rank Order/Pearson Product Moment correlation, $R_{51-12} = -0.265$ to 0.106 , $P = 0.102-0.878$).

Occurrence of different forms of sexual activity

To get an indication if the three different states of sexual activity (SA⁻, SA⁺, and SA⁺⁺) are clearly distinct, or whether the SA⁺ condition represents a temporarily more limited transition-state between SA⁻ and SA⁺⁺, each observation of the complete observational data set on sexually active individuals between 1999 and 2003 ($n = 1662$) was categorized as belonging to either SA⁻, SA⁺ or SA⁺⁺ (see Fig. 4). The data clearly show that most observations within the group of sexually active individuals were done on bulls showing either no signs (SA⁻), or both

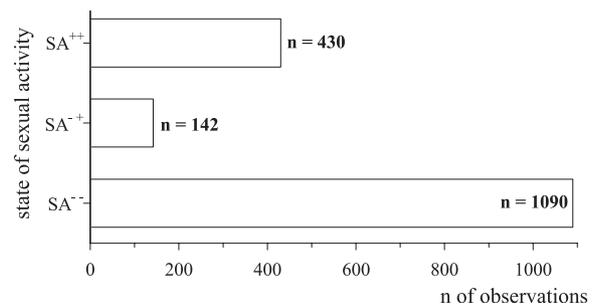


Fig. 4. Horizontal bar plot of the complete observational data set categorized to the number of observations within each of the three different states (SA⁻, SA⁺, and SA⁺⁺) of sexual activity.

TGS and UD (SA⁺⁺). Least recorded were SA bulls showing additionally TGS only (SA⁻⁺). Although some of the samples in the SA⁻⁺ group came from bulls later entering SA⁺⁺ and could thus be viewed as transition, some bulls had periods in SA⁻⁺ without showing UD at any point within that SA period.

Discussion

This study presents the first longitudinal data on individual hormone excretion patterns in free-ranging adult African elephant bulls, and examines endocrinological differences between qualitatively defined categories of sexual activity. The results indicate that measurements of fecal epiandrosterone (EA) and 3 α ,11oxo-cortisol metabolites (3 α ,11oxo-CM) generate useful information on androgen and glucocorticoid status in elephant bulls under free-ranging conditions, as recently described for animals in captivity (Ganswindt et al., 2002, 2003). Androgen levels were generally elevated during sexually active periods in which TGS and/or UD occurred, whereas sexually active periods, regardless of the presence or absence of these signals, were not linked to elevations in glucocorticoid levels.

A positive correlation between age and the occurrence of TGS and UD has been shown to exist in sexually active bulls (Poole, 1987, 1989b). The present study confirms this, but also demonstrates that the elevation in androgen levels seen in bulls showing either sign was unrelated to age, indicating that the presence of the signal, rather than age, is the main predictor of increased androgens. Thus, by showing that younger musth bulls, at least with respect to EA levels are not false signaling, our results support the earlier predictions of Poole (1989a) that musth is an honest signal.

Although EA concentrations were significantly higher in bulls showing TGS compared to bulls without TGS, the signal strength was not related to androgen level, irrespective of whether or not UD was present. On this basis, we propose four “reproductive” categories of adult bull elephant; three sexually active (SA only, SA plus TGS, and SA plus TGS and UD) and one sexually inactive. Among the SA bulls, the TGS only group occurs at a relatively low frequency and had only a 2-fold increase in EA levels compared to a 4-fold increase in the group with UD, suggesting that the TGS only group may represent more a transitional condition between the other two groups, rather than a separate and distinct state. Thus, the two sexually active states, musth and non-musth, seem to be “on/off” states rather than a continuous change linked to age. However, further analysis, preferably of longitudinal data, would be needed to clarify if this is so and whether, in fact, bulls do spend extended periods in this state without ever progressing to the stage of showing both TGS and UD. On the other hand, the possibility that additional stages of SA

also exist cannot be excluded from our data. Variation in androgen levels among bulls showing physical signs was considerably higher than in the other groups and this may be an indication that other factors, such as sustained close proximity to an estrus female (consortship) or having experienced a fight, could influence androgen status in these animals.

The only other study describing quantitative differences in testosterone levels according to reproductive state (Poole et al., 1984), also demonstrated an elevation of testosterone levels in active non-musth bulls compared to the group of sexually inactive individuals, but since sample size was low and no clear definition of the state of sexually active non-musth was given, direct comparison of results is difficult. Nevertheless, the findings of both studies indicate that within the reproductive context of male African elephants, at least two distinct states of sexual activity exist and that these clearly differ in the occurrence of the musth-typical signs (TGS and UD). From the present study, we can now show that these two states also differ in terms of androgen levels. Sexually active bulls with TGS and UD had the highest androgen levels, providing a likely physiological explanation for the observation that this category of bulls also exhibited the highest rates of aggression (Poole, 1989a), since androgenic steroid hormones are known to mediate aggressive behavior in diverse mammalian species (Brain, 1979; Leshner and Moyer, 1975; Ogawa et al., 1996; Rose et al., 1974). The simultaneous occurrence of elevated androgens, high levels of aggression and therefore increased likelihood of engaging in physical fights in SA bulls showing TGS and UD (Hall-Martin, 1987; Poole, 1989a) indicates that this state should be viewed as a competitive strategy.

On the basis of this, we suggest that only this competitive part of African bull sexual activity should be called “musth” and not the whole complex of sexual activity, hence supporting (Poole, 1987) original definitions. The bulls in the TGS only group may then represent bulls “primed” for a competitive mode with the local social environment determining if they proceed to become fully competitive. For example, our observations showed that bulls can switch urine dribbling on (i.e., enter musth) within hours of finding an estrous female and switch it off (i.e., go out of musth) within minutes of arrival of a much higher ranking musth bull, whereas TGS continued to be seen for several days after UD had ceased.

In contrast to the differences in EA levels during different states of sexual activity, this study indicates that sexual activity in free-ranging African elephants is not associated with marked elevations in glucocorticoid output. No differences in fecal 3 α ,11oxo-CM levels were found between the three states of sexual activity, nor between states of sexual activity and inactivity. These results conform to those of a recent study in captive elephants (Ganswindt et al., 2003), which also revealed no elevation of cortisol metabolites during the competitive state of musth.

On an individual basis, any elevation in glucocorticoids that may occur would be most likely to be seen towards the end of the reproductively active periods when loss of condition and energetic stress may be highest. However, as indicated in the two profiles shown in Fig. 2, no change in glucocorticoids could be detected during the entire course of these sexually active periods, independent of whether or not they were associated with musth (SA⁺⁺).

Confirmation that the measurements obtained with our 3 α ,11oxo-CM assay do, in fact, reflect adrenal glucocorticoid output is provided by the clear (5- to 10-fold) increase in levels found in two bulls following severe physical injury (broken leg and gunshot wounds) (data not shown). Thus, based on fecal glucocorticoid profiles in both captive (Ganswindt et al., 2003) and wild animals (present study), available information provides no indication that musth in the African elephant represents a physiological stress associated with activation of the hypothalamic–pituitary–adrenal axis (HPA axis). Since, however, it is known that stress-related factors independent of enhanced adrenal activity, such as increased secretion of endogenous opioid peptides (e.g., Abbott et al., 1997) can be involved in stress response in mammals, further studies are needed to explore other metabolic pathways that could respond to the potential stressful condition of musth. Further, the possibility of a suppressive effect of elevated androgen levels on adrenocortical activity in mammals (Kenagy et al., 1999) through mechanisms involving enhanced glucocorticoid feedback regulation (Viau and Meaney, 2004) may have obscured any stress-related adrenal response. Ecological factors such as food availability and season-dependent rainfall, which have been associated with increased adrenal endocrine function in female elephants (Foley et al., 2001), should also be considered. Finally, it would be useful to examine whether potential stress-inducing parameters, such as increased restlessness, reduced feeding activities, or increased inter-male aggression (Hall-Martin, 1987; Poole, 1989a), are only associated with musth or whether they also occur in other sexually active states.

In conclusion, we have shown that elevated levels of androgens during sexually active periods are only seen in bulls showing TGS and UD and that these elevated levels appear to be irrespective of the age of the individual, hence supporting that musth is an honest signal. Based on this, we suggest that the term “musth” should be exclusively linked to the competitive state in sexually active male elephants and that the presence of urine dribbling should be the physical signal used for defining this state. Further, we have for the first time obtained results on glucocorticoid levels in free ranging elephant bulls. These results indicate that reproductively active periods, both musth and non-musth, do not seem to stimulate HPA axis activity. Further studies are, however, necessary to understand the functionality of the different forms of sexual activity and their potential impact on reproduction in free-ranging African elephants.

Acknowledgments

We thank the Office of the President of the Republic of Kenya, Kenya Wildlife Service, Samburu County Council, Isiolo County Council, and reserve wardens for permission to work in the Samburu and Buffalo Springs National Reserves. The Evangelisches Studienwerk e.V. Villigst and the International Elephant Foundation for financial support, and the staff of Save the Elephants for organizational help and sample collection. We also thank Dr. R. Palme and Dr. E. Möstl for providing access to the reagents of the 3 α ,11oxo-CM EIA. Finally, we thank A. Heistermann and J. Hagedorn for expert help in laboratory techniques.

References

- Abbott, D.H., Saltzman, W., Schulz-Darken, N.J., Smith, T.E., 1997. Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. *Ann. N.Y. Acad. Sci.* 807, 219–238.
- Barnes, R.F.W., 1982. Mate searching behavior of 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.
- Eggeling, H., 1901. Über die Schilfdrüse des Elefanten. *Biol. Centralblatt* 21, 443–453.
- Foley, C.A.H., Papageorge, S., Wasser, S.K., 2001. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. *Conserv. Biol.* 15, 1134–1142.
- Ganswindt, A., Heistermann, M., Borragan, S., Hodges, J.K., 2002. Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. *Zoo Biol.* 21, 27–36.
- Ganswindt, A., Heistermann, M., Palme, R., Borragan, S., Hodges, J.K., 2003. Non-invasive assessment of adrenal function in the male African elephant (*Loxodonta africana*) and its relation to musth. *Gen. Comp. Endocrinol.* 134, 156–166.
- Hall-Martin, A.J., 1987. Role of musth in the reproductive strategy of the African elephant (*Loxodonta africana*). *S. Afr. J. Sci.* 83, 616–620.
- Holm, S., 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* 6, 65–70.
- Huber, S., Palme, R., Arnold, W., 2003. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). *Gen. Comp. Endocrinol.* 130, 48–54.
- Jachmann, H., 1985. Estimating age in African elephants. *Afr. J. Ecol.* 23, 199–202.
- Jachmann, H., 1988. Estimating age in African elephants: a revision of Law's molar evaluation technique. *Afr. J. Ecol.* 26, 51–56.
- Kahl, M.P., Armstrong, B.D., 2002. Visual displays of wild African elephants during musth. *Mammalia* 66, 159–171.
- 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. Endocrinol.* 115, 236–243.
- Laws, R.M., 1966. Age criteria for the African elephant, *Loxodonta africana*. *E. Afr. Wildl. J.* 4, 1–37.
- 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.
- Möhle, U., Heistermann, M., Palme, R., Hodges, J.K., 2002. Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male nonhuman primates. *Gen. Comp. Endocrinol.* 129, 135–145.

- Moss, C.J., 1996. Getting to know a population. In: Kangwana, K.F. (Ed.), *Studying Elephants*. African Wildlife Foundation, Nairobi. Chapter 7.
- Moss, C.J., Poole, J.H., 1983. Relationships and social structure of African elephants. In: Hinde, R.A. (Ed.), *Primate Social Relationships: An Integrated Approach*. Blackwell Scientific Publications, Oxford, pp. 315–325.
- Möstl, E., Maggs, J.L., Schrötter, G., Besenfelder, U., Palme, R., 2002. Measurement of cortisol metabolites in faeces of ruminants. *Vet. Res. Com.* 26, 127–139.
- Ogawa, S., Robbins, A., Kumar, N., Pfaff, D.W., Sundaram, K., Bardin, C.W., 1996. Effects of testosterone and 7 α -methyl-19-nortestosterone (MENT) on sexual behaviors in two inbred strains of male mice. *Horm. Behav.* 30, 74–84.
- Omond, P., Bitok, E., Kahindi, O., Mayienda, R., 2002. Total Aerial Count of Elephants in Samburu/Laikipia. Kenya Wildlife Service Report, Nairobi.
- Palme, 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*. Akadémiai Kiadó, Budapest, pp. 111–117.
- Poole, J.H., 1982. Musth and male–male competition in the African elephant. PhD Thesis, University of Cambridge, UK.
- Poole, J.H., 1987. Rutting behavior in African elephants: the phenomenon of musth. *Behavior* 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 behavior of African elephants. In: Short, R.V., Balaban, E. (Eds.), *The Differences between the Sexes*. University Cambridge, pp. 331–346.
- Poole, J.H., Moss, C.J., 1981. Musth in the African elephant. *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.
- Raeside, J.I., Renaud, R.L., Marshall, D.E., 1992. Identification of 5 α -androsterone-3 β , 17 β -diol and 3 β -hydroxy-5 α -androsteran-17-one sulfates as quantitatively significant secretory products of porcine Leydig-cells and their presence in testicular venous-blood. *J. Steroid. Biochem.* 42 (1), 113–120.
- Rasmussen, L.E.L., Schulte, B.A., 1998. Chemical signals in the reproduction of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. *Anim. Reprod. Sci.* 53, 19–34.
- 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., 2004. Estimating age of immobilized elephants from teeth impressions using dental silicon. *Afr. J. Ecol.* (submitted for publication).
- 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.
- 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.
- Wasser, S.K., Risler, L., Steiner, R.A., 1988. Excreted steroids in primate feces over the menstrual cycle and pregnancy. *Biol. Reprod.* 39, 862–872.
- Whitehead, H., 1994. Delayed competitive breeding in roving males. *J. Theor. Biol.* 166, 127–133.
- Wittemyer, G., 2001. The elephant population of Samburu and Buffalo Springs National Reserves, Kenya. *Afr. J. Ecol.* 39, 357–365.
- Ziegler, T., Hodges, J.K., Winkler, P., Heistermann, M., 2000. Hormonal correlates of reproductive seasonality in wild female Hanuman Langurs (*Presbytis entellus*). *Am. J. Primatol.* 51, 119–134.