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Chemosignalling of musth by individual wild African elephants (*Loxodonta africana*): implications for conservation and management

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Elephants have extraordinary olfactory receptive equipment, yet this sensory system has been only minimally investigated in wild elephants. We present an in-depth study of urinary chemical signals emitted by individual, behaviourally characterized, wild male African elephants, investigating whether these compounds were the same, accentuated, or diminished in comparison with captive individuals. Remarkably, most emitted chemicals were similar in captive and wild elephants with an exception traced to drought-induced dietary cyanates among wild males. We observed developmental changes predominated by the transition from acids and esters emitted by young males to alcohols and ketones released by older males. We determined that the ketones (2-butanone, acetone and 2-pentanone, and 2-nonanone) were considerably elevated during early musth, musth and late musth, respectively, suggesting that males communicate their condition via these compounds. The similarity to compounds released during musth by Asian male elephants that evoke conspecific bioresponses suggests the existence of species-free 'musth' signals. Our innovative techniques, which allow the recognition of precise sexual and musth states of individual elephants, can be helpful to managers of both wild and captive elephants. Such sampling may allow the more accurate categorization of the social and reproductive status of individual male elephants.

Keywords: ketones; cyanates; social status; communication; olfaction

1. INTRODUCTION

Coordinated behavioural and chemical studies of captive and semi-wild Asian elephants (*Elephas maximus*) have demonstrated that specific compounds facilitate important functional social roles throughout life (Rasmussen *et al.* 1997*b*; Rasmussen & Schulte 1998, 1999; Rasmussen & Krishnamurthy 2000). Wild Asian elephants produce and respond to similar chemical signals (Rasmussen *et al.* 1997*b*; Rasmussen & Krishnamurthy 2000), utilizing their well developed olfaction and vomeronasal organ systems (Rasmussen 1999*a*; Lazar *et al.* 2000). By contrast, olfactory-based behaviours have not been rigorously studied in captive African elephants (*Loxodonta africana*), although observations of such behaviours have been recorded among wild elephants (Douglas-Hamilton 1972; Moss 1983; Poole 1987, 1989). In addition, chemical identification of compounds functioning as signals in this species lags behind information on the Asian species (Buss *et al.* 1976; Wheeler *et al.* 1982; Rasmussen *et al.* 1996*a*; Goodwin *et al.* 1999). This paper, we believe, presents the first investigation of chemical signals in a wild African elephant population.

A database of urinary volatile compounds has been complied from more than 100 samples collected from captive elephants of known sex, age, dominance status and measured hormonal and behavioural condition. Although this database is primarily on Asian elephants, recent studies have included a number of African elephants (Rasmussen *et al.* 1996*a*; Goodwin *et al.* 1999; Riddle *et al.* 2000), thus allowing comparison between the two species. The initial field investigation focused on urinary volatile compounds for two reasons. First, elephants heavily utilize the medium of urine, especially urinary volatiles, to broadcast chemical signals (Rasmussen *et al.* 1982, 1986, 1996*b*; Rasmussen 1998, 1999*b*; Rasmussen & Krishnamurthy 2000). Responses involving paired categories of conspecifics have been documented (Rasmussen *et al.* 1997*b*; Rasmussen & Schulte 1999; Schulte & Rasmussen 1999*b*). Second, fresh urine samples were obtainable after observations of specific behaviours, followed by urination, or after urine expression, followed by an elicited response by conspecifics.

The individually identified elephants of the Samburu and Buffalo Springs National Reserves, Kenya (Wittemyer 2001) offer a unique opportunity to characterize chemical signals in a wild population of African elephants. The elephants in this area are partially habituated to human presence, allowing behavioural observations to be conducted easily and without disturbance to the focal individuals. Musth cycles of the dominant male elephants in the population have been recorded since November 1997. Information on the length and timing of musth not only gives direct information on musth itself, but also allows the assessment of the relative social status of the males studied in this investigation (Poole 1987). These comprehensive background records make this elephant population a valu-

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able resource for the study of chemical signals in the African elephant.

Because initial wild studies (Rasmussen *et al.* 1996*a*) and longer-term captive studies clearly demonstrated chemical differences between musth and non-musth male elephants (Perrin *et al.* 1996; Rasmussen & Perrin 1999; Schulte & Rasmussen 1999*a*), we chose to focus on a comparison of musth and non-musth males. Additionally, sampling of a demographic cross-section of the male population was conducted. This included a seven-year-old male, four adolescent males (ages 15–25 years), and eight mature males (ages 26–43 years), four of which were over the age of 35 years and considered socially dominant. All elephants were individually recognized, and their sexual state, social grouping and status, both at the time of sampling and for several years previously, was characterized. This study presents, to our knowledge, the first detailed comparison of urinary chemical signals in captive and wild male African elephants, during non-musth and musth. We were interested whether chemical signals were the same, accentuated or diminished in this free-ranging population compared with captive groupings and whether chemical profiles were indicative of the age and musth status of individuals. Interspecies comparison between African and Asian male elephant chemical profiles is also presented.

2. METHODS

(a) *Enumeration of urinary samples*

The ability to individually recognize each wild male elephant with information on his age, history, relative status and physiological condition contributed to the uniqueness of this study. In the Samburu study area, we obtained 23 samples from 13 male African elephants. We compared these volatile urine samples and surrounding background air samples with 11 samples collected from four African male elephants living in captivity in the USA. Our specimens from the wild included those obtained from a seven-year-old male, four adolescent males (ages 15–25 years) and eight mature males (ages 26–43 years) (table 1).

Samples were further categorized according to the stage of maturity and/or physiological state of the elephant. The mature males included pre-musth, musth, post-musth, and non-musth animals. Non-musth individuals had shown no signs of musth for at least two months; pre-musth animals demonstrated erratic urine dribbling and posturing with slightly swollen temporal glands; musth males demonstrated marked temporal gland swelling and secretion, characteristic posturing, urine dribbling and a green penis (Poole 1987); post-musth individuals were sampled within the two month period after the cessation of visible signs of musth.

The relative rank of the five oldest males (B1001, B1007, B1009, B1011, and B1033) was difficult to estimate as the males generally avoided overlapping their musth periods. Observations during non-musth periods indicated that B1001 and B1009 were dominant to B1011 (table 1).

(b) *Field collection procedures*

Field collection procedures involved: (i) individually identifying a focal elephant and its immediate neighbours; (ii) observing their behaviours to identify their sexual condition, particularly that of the focal animal; (iii) discretely tracking until the elephant urinated; and (iv) collection of the sample for subsequent chemical analyses. The time interval for steps (ii) and (iii) varied between 15 min and 6 h, but samples of urinary volatiles were collected immediately after urination (1–2 min). The volatile compounds (i.e. low molecular weight, gaseous compounds) of urine were captured as headspace samples in evacuated, specially designed stainless steel containers by two methods.

(i) If sufficient urine remained above ground, a stainless steel measuring cup was used to carefully scoop 50 ml of urine into a clean, squat 250 ml glass vial. Subsequent procedures to capture the contained headspace (CHS) volatiles into evacuated containers were slight modifications of the procedure used under laboratory conditions in the USA (Rasmussen & Perrin 1999). Slightly higher temperatures and longer equilibration/sampling times were employed in the field.

(ii) If it was not possible to obtain liquid samples, an inverted funnel attached to the evacuated bottle was placed *in situ* firmly over the recently voided urine sample on the ground. After equilibration, the drawing of headspace volatiles into the evacuated container was conducted in a slow, steady manner to trap the compounds volatilizing from the fallen urine. These are presumably the same compounds that adjacent elephants detect by olfaction and/or by their vomeronasal organ as evidenced by sniffing, flehmens or other observed behaviours.

Our method allows long-term storage at ambient temperature of these elephant odours until gas chromatographic/mass spectrometric (GC/MS) analyses. Further details are provided in Perrin *et al.* (1996) and Rasmussen & Perrin (1999). Background air samples were gathered as controls. We obtained sufficient duplicate samples, either by the same method or by the two slightly different techniques, to allow inter- and intra-method comparisons, as this was the first time field sampling of African elephant odours has been attempted by these headspace capture methods. Duplicate *in situ* and triplicate CHS samples from a single urination (B1045) are used for this comparison. The collection of several discrete samples during varying physiological conditions from individual males allowed analysis of the variability in individual chemical profiles across states (table 1).

(c) *Qualitative and quantitative analytical procedures for urinary volatiles*

The total non-methane hydrocarbon concentration was measured to allow quantitative comparison between samples (Rasmussen & Perrin 1999). Then GC/MS analyses were conducted on equivalent aliquots of samples by the methods described in detail in Rasmussen & Perrin (1999) and Perrin *et al.* (1996). The mass spectrometer was programmed for a mass scan of 33–300, which allowed for identification of compounds from C3 through to C14. The conditions allowed quantitation as low as 0.10 ppbv (parts per billion volume). Compounds were identified using an NBS 75 K Hewlett-Packard MS Chem Station library search and were manually rechecked with the NIST/EPA/NIH Mass Spectral Data Base v. 4.01, the Wiley library and our library of elephant-specific compounds from elephants. Compounds of specific interest (12 ketones, 5 alcohols, and several cyanates and isothiocyanates) were also analysed as authentic synthetic compounds to allow positive identification and quantitation in comparison with these standards.

Table 1. Urinary samples from wild male African elephants.
(Definition and abbreviation: *in situ*, sample obtained via inverted funnel placed tightly over urine spot on ground; CHS, contained headspace sample.)

date	urine	ID	estimated age (yr)	state of maturity	musth
2/3/99	*in situ*	BSOC	7	juvenile	non-musth
17/3/99	*in situ*	B1045[a]	20	adolescent[a]	non-musth
17/3/99	CHS	B1045[a]	20	adolescent	non-musth
17/3/99	CHS	B1077[b]	19	adolescent[b]	non-musth
7/3/99	*in situ*	B1073	21	adolescent	non-musth
7/3/99	*in situ*	B1039[c]	25	mature[c]	non-musth
1/7/99	*in situ*	B1042[d]	26	mature[d]	pre-musth
10/7/99	*in situ*	B1042[d]	26	mature	musth
5/7/00	*in situ*	B1042[d]	27	mature	musth
6/6/00	CHS	B1029[e]	28	mature	post-musth[e]
3/8/00	CHS	B1029	28	mature	non-musth
31/5/00	CHS	B1019[f]	30	mature	post-musth[f]
4/8/00	CHS	B1019[f]	30	mature	non-musth
31/7/00	CHS	B1011	38	mature	non-musth
1/4/99	*in situ*	B1001[g]	43	mature[g]	musth
31/7/00	CHS	B1009	40	mature	non-musth
4/8/99	CHS	B1007[h]	41	mature[h]	pre-musth
11/8/99	*in situ*	B1007[h]	41	mature	musth[h]
10/7/00	CHS	B1033[i]	40	mature	post-musth[i]

[a] Five samples. No musth episodes observed.
[b] Two samples. No musth episodes observed.
[c] One sample. Observed sexually investigating females, musth episodes observed.
[d] Three samples. Since 1998, recorded to come into musth for approximately one month (July). The sample on 1st July 1999 was during early musth; the sample on 10th July 1999 was during full musth; the sample on 5th July 2000 was during full musth.
[e] Two samples. Taken approximately one month post-musth; seen in musth May 2000.
[f] Two samples. Post-musth sample taken on 31st May 2000 after seen in musth on 11th May 2000 (two weeks after musth cessation).
[g] One sample. During 1998, his musth episode lasted two months and in 1999 it lasted three months (similar to captive lengths). This bull did a flehmen to his own expressed urine. On human skin, unpleasant odoriferous urinary components were detectable for 24 h, despite repeated washings with soap.
[h] Two samples. During 1998, his musth period lasted for 4–5 months; the sample on 4th August 1999 was during pre-musth; the sample on 11th August 1999 was during early urine dribbling period.
[i] One sample. Obtained one month post-musth.

Table 2. Compound concentrations and collection methodology. Concentration (in $\mu g\ m^{-3}$) of five principal urinary volatiles.
(For all pairs there was no statistical difference between two groups.)

compound	*in situ*	CHS	statistical results
acetone	14.9 ± 0.05	11.0 ± 1.64	$t = 1.81$; d.f. = 3; $p = 0.169$
2-butanone	0.77 ± 0.16	1.27 ± 0.27	$t = -1.39$; d.f. = 3; $p = 0.2594$
2-pentanone	0.23 ± 0.06	0.55 ± 0.11	$t = -2.14$; d.f. = 3; $p = 0.1220$
2-propanol	1.35 ± 0.12	2.01 ± 0.51	$t = -0.996$; d.f. = 3; $p = 0.3925$
3-hexen-1-ol	0.13 ± 0.02	0.17 ± 0.02	$t = -1.37$; d.f. = 3; $p = 0.2635$

Sample sizes were limited in this initial study because of the exploratory nature and the logistics involved in the collection and transfer of the samples to the laboratory. Parametric statistical methods, *t*-tests, were used only in tables 2 and 3 to assess the difference between concentrations collected *in situ* and by the CHS method. Because wild individuals were sampled multiple times, in various states and across different years, analyses of the compound concentrations and presence between various states were complicated and the use of statistical evaluation was limited (Sokal & Rohlf 1995; Lehner 1996). Samples from the same individuals could not be treated as independent, resulting in too few samples for quantitative analyses. We present qualitative results on compound concentrations across different physiological states. In tables 4 and 5, we have presented evident differences in compounds across varying states.

3. RESULTS

(a) *Comparison of urinary volatiles collected by two headspace methods*

No significant differences in concentration were observed for five compounds measured in duplicate samples utilizing the two collection methods (table 2). Acetophenone concentrations ($0.26 \pm 0.05\ \mu g\ m^{-3}$) in triplicate CHS samples demonstrated no intracompound differences. Comparison of urinary levels of six ketones

Table 3. Compound concentrations and collection methodology. Concentrations of 10 compounds in duplicate CHS samples.

compounds from B1077 (non-musth samples)	aliquot 1 (μg m^{-3}) (2–4 h)	aliquot 2 (μg m^{-3}) (4–6 h)
acetone	16.84	14.32
2-butanone	1.01	0.88
2-propanone	2.37	1.79
3-hexanone	0.12	0.06
3-octanone	0.72	0.49
2-octanone	0.34	0.06
acetophenone	0.27	0.09
2-propanol	3.11	1.92
3-hexen-1-ol	0.06	0.21
2-ethyl-1-hexanol	0.77	0.23

Table 4. Urinary compounds in mature wild African male elephants: comparison of varying musth states (median 75–25%).[a]

compounds	pre-musth $n = 2$	musth $n = 4$	post-musth $n = 3$	non-musth $n = 4$
esters	0, 2	0–1	0–1	0–4
ketones	31.5 (35.0–28.0)	33.0 (51.5–16.0)*	27.0 (28.5–21.8)	12.5 (14.0–9.0)*
alcohols	5.0 (10.0–0.0)	7.0 (13.5–5.0)	4.0 (8.5–4.0)	3.5 (7.0–3.0)
aldehydes	4.5 (9.0–0.0)	11.0 (11.8–4.3)	5.5 (8.0–3.5)	11.5 (13.0–8.5)
sulphur-C	4.5 (9.0–0.0)	2.5 (3.5–1.0)*	4.0 (4.8–1.0)	7.0 (7.0–6.0)*
phenols	3.5 (4.0–3.0)	1.5 (3.5–0.0)	0.0 (2.3–0.0)	1.0 (1.5–0.5)
acids	0.5 (1.0–0.0)	1.5 (3.0–0.5)	1.0 (1.8–1.0)	3.0 (4.0–2.5)
cyanates[b]	0	2.0 (2.0–2.0)	5.0 (7.3–2.8)	6.0 (6.5–3.5)
bicyclo	6, 15	5.0 (6.5–3.5)*	6.0 (5.0–4.5)	1.5 (3.0–0.0)*
methyl substituted-cyclohexenones	5.0 (5.0–5.0)	4.0 (4.0–4.0)*	4.0 (4.75–1.0)	0.0 (0.0–0.0)*
farnesols			1	0, 2

[a] As the sample sizes are a mix of different and same males and affected by pseudoreplication, all samples cannot be considered independent. Unconfounded samples were too few for statistical analyses. The interquartile range of the musth and non-musth compounds, indicated by an asterisk, does not overlap.

[b] Compounds included: methane isocyanato, methyl cyanide, thiocyanic acid, 1-isocyanato butane, methane isothiocyanato, ethane isothiocyanato, thiocyanic acid ethyl ester, isopropyl isothiocyanate, 2-isothiocyanato-2-methyl propane, 2-isothiocyanato butane and isobutyl isothiocyanate; most contained methyl cyanide.

and three alcohols in two successive *in situ* samples demonstrated higher concentrations in the first aliquot (2–4 h post-sampling) than the second (4–6 h post-sampling), although the statistical significance of the difference could not be assessed due to the small sample sizes (table 3).

(b) *Qualitative comparison of urinary volatiles*

(i) *Captive and wild condition*

The types of compounds and the individual compounds identified in the volatile headspace of urine were similar for both wild and captive African elephants (table 4). Qualitative differences were found between the two in furans, sesquiterpenes (tri/bicyclic compounds) and cyanates. Noteworthy are the large numbers of cyanates and isothiocyanates in the wild elephants compared with a single cyanate detected in a captive animal.

(ii) *Musth and non-musth conditions*

The physiological condition of the males, either musth or non-musth, was reflected in the urinary compounds. The urine of wild musth males contained greater numbers of ketones, alcohols, and substituted cyclohexenones, than did the urine of non-musth males which contained more esters and acids.

Differences in types of chemical compounds were especially noteworthy in wild samples from the four physiological stages of musth categorized in this study: non-musth, pre-musth, musth, and post-musth (table 4). For aldehydes and alcohols, minor differences were apparent across these four categories (table 4). However, individual aldehydes and alcohols demonstrated concentration differences across the states. The total numbers of ketones present in musth urine were twofold those in non-musth urine. Five or more methyl-substituted cyclohexenones were present that were not detected in non-musth urine. A total of 11 different cyanates and isothiocyanates were identified, with a single sample from a post-musth male containing 8 of the 11 compounds. The urine from the youngest wild elephant and the non-musth captive animals did not contain any cyanates, and the number of different cyanates was low in the younger wild males.

(c) *Quantitative comparison of selected volatiles*

Our captive studies demonstrated qualitative and quantitative variations in urinary volatiles between musth and non-musth African elephants (Rasmussen *et al.* 1996*a*, L. E. L. Rasmussen and T. E. Goodwin, unpublished data). Utilizing this captive database of compounds as a guide-

Table 5. Urinary compounds demonstrating concentration differences among male African elephants of various ages and states (μg m^{-3}) (median 75–25%).
(Because some samples were taken deliberately from the same individual, either in the same state or a different state, it is not possible with the small numbers of samples and individuals to perform meaningful statistical tests. The most apparent large concentration differences between states are listed in this table and figure 1.)

compound	state	second state
acetone	wild musth, 28.8 (23.3–44.4)	wild post-musth, 6.55 (4.89–8.02)
	wild musth, 28.8 (23.3–44.4)	wild non-musth, 13.7 (12.3–15.4)
	captive non-musth, 8.31 (8.16–8.92)	wild non-musth, 13.7 (12.3–15.4)
2-butanone	wild pre-musth, 6.95 (5.1–8.7)	wild post-musth, 0.62 (0.29–1.23)
2-pentanone	wild musth, 2.02 (1.28–5.47)	wild non-musth, 0.15 (0.07–0.29)
	wild musth, 2.02 (1.28–5.47)	wild pre-musth, 0.125 (0.12–0.13)
	wild musth, 2.02 (1.28–5.47)	wild post-musth, 0.11 (0.09–0.19)
3-octanone	wild musth, 0.91 (0.73–1.26)	wild non-musth, 0.07 (0.00–0.23)
2-nonanone	wild musth, 1.23 (1.00–2.96)	wild non-musth, 0.00 (0.00–0.00)
	wild musth, 1.23 (1.00–2.96)	wild post-musth, 0.02 (0.005–0.043)
6-methyl-2-heptanone	wild musth, 0.27 (0.25–0.42)	wild non-musth, 0.08 (0.00–0.13)
2-propanol	non-musth (<19 years), 1.69 (1.35–2.23)	non-musth (>20 years), 0.46 (0.39–0.73)
2-ethyl-1-hexanol	non-musth (all), 0.11 (0.00–0.31)	musth, 0.85 (0.65–0.89)

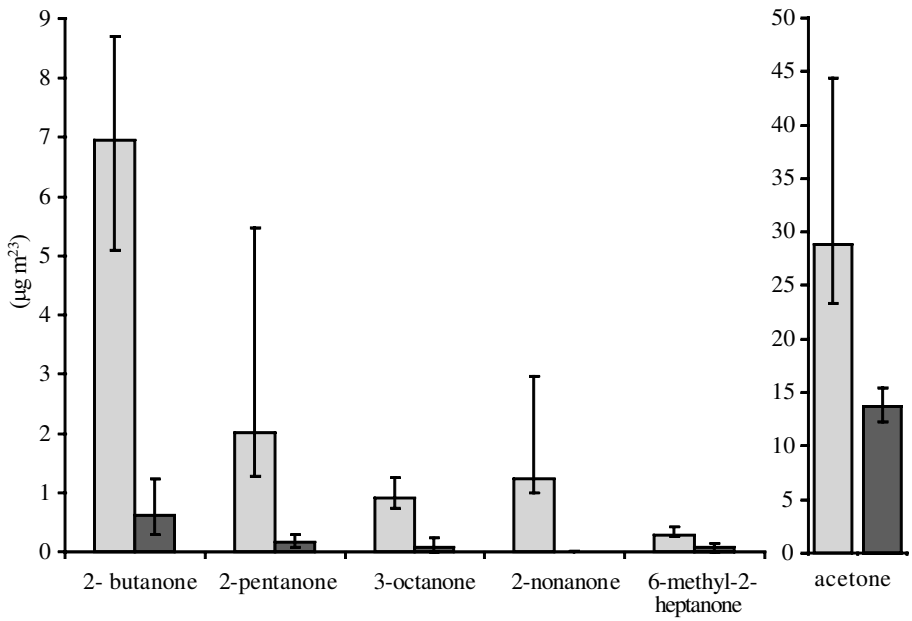

Figure 1. Musth (light grey bars) and non-musth (dark grey bars) concentrations of five urinary ketones from wild African male elephants. Columns indicate medians and error bars interquartile ranges.

line and adding new compounds identified in our wild elephant samples, 16 urinary compounds were quantified in 13 male African elephants. Table 5 contains median and interquartile ranges for compound concentrations for designated sexual states. The statistical significance of the differences could not be assessed due to the limitations of sample sizes.

(i) *Ketones*

Among ketones, urinary acetone concentrations indicated musth or non-musth states in the wild male African elephants. Among non-musth wild males, no significant concentration differences were observed in any age categories. In captive non-musth males, acetone was lower than in wild males. However, in both wild and captive males, acetone concentrations were greater during musth than during non-musth or post-musth (table 5; figure 1). No concentration differences were observed between captive and wild musth males.

2-butanone concentrations were higher in pre-musth samples than in post-musth (table 5; figure 1), although one 28-year-old male (B1029) demonstrated the highest concentration (1.43 μg m^{-3}). There were no apparent differences between musth, non-musth, or post-musth samples. In non-musth males, not only were there no large differences among age classes for wild elephants, but wild and captive levels of 2-butanone were similar.

A third ketone, 2-pentanone, showed much higher concentrations during musth than in non-musth, pre-musth or post-musth (table 5; figure 1). Exceptionally, the two samples from the 19-year-old non-musth male (B1077) contained the highest 2-pentanone concentrations at 1.79 μg m^{-3} and 9.37 μg m^{-3}.

2-nonanone concentrations during musth were much greater than concentrations in post-musth (table 5; figure 1). The levels were undetectable in pre-musth males, mostly undetectable in non-musth males, and at low levels in post-musth males. Interestingly, the values for the five musth bulls were 0.68, 1.11, 1.23, 2.57 and 4.11 $\mu g\ m^{-3}$. This variation is commented on in § 4.

Seven urinary ketones (3-hexanone, 2-hexanone, 2-ethyl-2-pentanone, 4-heptanone, 2-heptanone, 3-heptanone, and 2-nonanone) had nondetectable or very low levels (nondetected or less than 0.08 $\mu g\ m^{-3}$) in non-musth wild elephants. In marked contrast, the concentrations of these seven urinary ketones and 3-octanone were sizeably higher in musth elephants (0.12–3.19 $\mu g\ m^{-3}$; figure 1). Only two deviations from this pattern were observed. B1077's non-musth concentration of 4-heptanone was 0.22 $\mu g\ m^{-3}$, near musth levels of other individuals. B1009's non-musth 3-heptanone concentration was 0.8 $\mu g\ m^{-3}$.

Most urine samples contained substantial levels of 6-methyl-2-heptanone (0.22–0.73 $\mu g\ m^{-3}$). In musth animals, the levels were considerably higher than in non-musth animals (table 5). Another ketone, acetophenone, was also present in most samples with no difference between various musth states.

(ii) *Alcohols*

Five alcohols exhibited characteristic concentration patterns related to age, maturity, and musth state. 3-hexen-1-ol was highest in young and teenage wild males, similar to results found with young Asian male elephants (see § 4). This alcohol was not detectable in older males, either during musth or non-musth. 2-propanol was also not detectable during musth or pre-musth, but post-musth samples contained this alcohol. 2-propanol was present in all non-musth samples and was much higher in young males than in older males. By contrast, while 2-ethyl-1-hexanol was not detected in the seven-year-old male and was sporadically detected in non-musth males, it was consistently present and higher in musth males (table 5). Two other alcohols, 2-butanol and 2,3,3-trimethyl oxetanol, were related to musth. The former demonstrated its highest levels during musth; the latter was not detectable in young and non-musth urine but was present at high levels in musth samples (0.33–2.33 $\mu g\ m^{-3}$).

(iii) *Cyanates and isothiocyanates*

Urinary cyanate concentrations differed with individual elephants. In March 1999 among three young males, one had low cyanate, a second had high isopropyl isothiocyanate (1.53 $\mu g\ m^{-3}$), and a third had high isothiocyanate methane concentrations.

Seasonal variations were apparent. From April through to August 1999, pre-musth and musth elephants demonstrated low amounts of several cyanates. A 41-year-old musth male was a notable exception; on 11th August, methyl cyanide concentration was 9.38 $\mu g\ m^{-3}$ whereas on 4th August (seven days earlier) no trace of methyl cyanide was apparent in the urine.

In the year 2000 (May to August), a remarkable difference was observed. Several isothiocyanate (especially isothiocyanatomethane) levels were greatly elevated. For example, B1019 (age 30 years, post-musth) demonstrated very high levels both of isothiocyanatomethane (63.55 $\mu g\ m^{-3}$) and isopropyl isothiocyanate (7.67 $\mu g\ m^{-3}$). Of the eight samples obtained in 2000, only one sample did not demonstrate these elevations.

4. DISCUSSION

The ability to capture chemical signals in the field from wild elephants and to couple sampling with behavioural observations and information on social status, age and physiological state of the sampled individual offers researchers new insight into the mechanisms by which male elephants govern their society and interact with females.

Several studies have demonstrated that wild and captive Asian elephants have a remarkably similar medley of volatile urinary compounds (Rasmussen & Krishnamurthy 2001). This intraspecies consistency is apparent in our current study of the elephants of the Samburu and Buffalo Springs National Reserves. Male African elephants of the same age and reproductive status present similar chemical profiles, whether in captivity or in the wild. The urine from both wild and captive animals contains a characteristic variety of volatile components; these include a spectrum of ketones, alcohols, aldehydes and sulphur-containing compounds. Such convergence indicates the strong influence of species specificity rather than dietary influence.

This study demonstrates the ability to chemically distinguish musth in male African elephants through analyses of urinary chemical signals. In captive male Asian elephants, characterization and quantitation of urine volatiles, in conjunction with serum androgen, metabolite measurements, and concurrent behavioural descriptions, have permitted the delineation of several stages of musth (Rasmussen & Perrin 1999). Using the captive chemical analyses from specific, behaviourally monitored field samples, chemical profiles were readily translated into meaningful and accurate information on stages of musth (Rasmussen & Krishnamurthy 2000; L. E. L. Rasmussen, V. Krishnamurthy and R. Sukumar, unpublished data). Although excellent behavioural data are available for some populations of wild African elephants (Moss 1983; Poole 1987, 1989), captive behavioural data concurrent with physiological and chemical studies, other than hormonal analyses, lag behind Asian studies. A database of urinary information from the captive population to compare with wild information is lacking for this species. Definitive information on chemical compounds relevant to stages of musth in African elephants is only available from studies of temporal gland secretions (TGS) (Rasmussen *et al.* 1996*a*). In this study, 16 compounds were identified in TGS during musth. Utilizing this information and information available from musth in Asian elephants, several observations on musth in the Samburu population of male African elephants can be made.

First, several wild male African elephants of the Samburu region, behaviourally described as pre-musth and exhibiting swollen temporal glands, demonstrated high urinary concentrations of 2-butanone. As was observed in Asian elephants, this suggests high levels of this compound are released, signalling impending musth. Second, urinary volatiles from African male elephants in musth are com-

posed of greater numbers and amounts of ketones than samples from non-musth elephants. Nine ketones were greater in musth than non-musth samples. Each ketone has a characteristic odour; it is probable that distinct chemical mixtures signify various sexual states, particularly musth. Especially interesting are the variable levels of 2-nonanone in four of the musth samples. In Asian elephants, 2-nonanone is highest at the urine dribbling or late part of musth. It is possible that the variable 2-nonanone levels are related to the stage of musth or even the social status of the sampled individual.

A possible correlation between male age and ketone and alcohol concentrations is suggested by comparisons between older and younger non-musth males and older and younger musth males. For the musth individuals, the two older males demonstrated higher concentrations of 3-hexanone, 2-hexanone, 2-ethyl-3-pentanone, 2-heptanone, 3-heptanone, 3-octanone, 2-nonanone and 2,3,3,-trimethyl oxetanol. Among the non-musth males, lower or non-detectable levels of two alcohols, 3-hexen-1-ol and 2-propanol, were found in the older males as compared with the younger males. Young male samples contained large amounts of sweet-smelling compounds like 3-hexen-1-ol and 2-propanol. These alcohols are reduced or not detected in the urine of adult musth males. Instead, the urine of these older musth males had sizeable amounts of 2,3,3-trimethyl oxetanol, not a sweet-smelling alcohol. Further studies involving greater numbers of elephants and samples will presumably reveal other differences. It is likely that the above chemical differences and others not yet detected will allow the detection of age, musth state, and social rank differences between males. One group of higher molecular weight alcohols, the farnesols, merit further intensive study as numerous substituted farnesols and, uniquely, their hydrates are present in African elephant exudates (Wheeler *et al.* 1982; Goodwin *et al.* 1999).

Only a few compounds—furans, sesquiterpenes, cyanates and/or isothiocyanates—were more varied in samples from wild compared with captive elephants. The more cosmopolitan diet of wild elephants probably influences their prevalence. Only in one captive sample were isopropyl isothiocyanate and methyl cyanide detected and these were in low concentration, whereas samples from wild elephants contained many cyanates and isothiocyanates, with especially high concentrations measured in samples collected in 2000, at the end of a nine month severe drought when food resources were limited (table 4). Thiocyanate synthesis is specific for the cyanide ion. Potentially, wild elephants eating organic cyanides would be exposed to toxic cyanide ions during thiocyanate formation with the potential for lethal toxicity (Merck Index), unless they have, like other herbivores, sufficient rhodanese enzyme to quickly convert the cyanides to thiocyanates (Bernfeld 1963). During this drought, several apparently healthy, normally behaving elephants died suddenly due to unknown causes. Surrounding the carcass of one such mature male was evidence of vomiting of partially digested plant matter. Unfortunately, limited resources did not allow confirmation of toxicity.

Chemical signals probably play dual roles by influencing spacing and instigating the suppression of musth states in submissive males (Rasmussen & Krishnamurthy 2000; Slotow *et al.* 2000). Musth males are prone to competitive interactions with other bulls that could potentially displace them as the primary breeding bull in an area. Although numerous musth bulls have been observed in the Samburu study area, rarely have two musth bulls overlapped in the area for more than a few days. Musth is an overt 'honest' behavioural signal but an energetically expensive one, indicating the willingness of a musth bull elephant to defend his sexual interests (Poole 1987, 1989). The primary communicatory signals used to announce the condition of musth include audition (musth rumbling), visualization (musth walk), and olfaction (altered secretion from temporal glands and urine dribbling) (Poole 1987, 1989; Rasmussen *et al.* 1996*a*). Pre-musth and musth urinary chemical signals (ketones and alcohols) overtly broadcast the long-lasting and distinct messages of musth. The chemicals released in urine undoubtedly communicate important behavioural cues regarding musth and thus the sexual state and status of a bull elephant. Such chemical cueing is potentially of great relevance to elephant reproductive behaviour and spacing.

This study demonstrates that the volatile chemical compounds in field-collected urinary samples can be utilized to distinguish musth from non-musth wild male African elephants and to distinguish stages of musth. Additionally, the similarity to compounds eliciting bioresponses in Asian elephants suggests species-free 'musth' signals. Potentially, urinary chemical signals can serve as a non-invasive technique for the assessment of sexual and social status with the possibility of using such chemical profiles to estimate age. To reach this level of refinement using this technique, greater numbers of samples need to be collected and analysed. By resolving whether a particular male is in pre-musth, musth or post-musth, research on breeding males can be conducted in greater detail. Such chemical information may eventually help managers to identify key breeding bull elephants within a population.

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