Sci Nat (2016) 103: 37
DOI 10.1007/s00114-016-1361-4

ORIGINAL PAPER

Reproductive endocrine patterns and volatile urinary compounds of *Arctictis binturong*: discovering why bearcats smell like popcorn

Lydia K. Greene[1,2] · **Timothy W. Wallen**[3,6] · **Anneke Moresco**[4,7] · **Thomas E. Goodwin**[3] · **Christine M. Drea**[1,2,5]

Received: 14 October 2015 / Revised: 22 March 2016 / Accepted: 27 March 2016 / Published online: 7 April 2016

Abstract Members of the order Carnivora rely on urinary scent signaling, particularly for communicating about reproductive parameters. Here, we describe reproductive endocrine patterns in relation to urinary olfactory cues in a vulnerable and relatively unknown viverrid—the binturong (*Arctictis binturong*). Female binturongs are larger than and dominate males, and both sexes engage in glandular and urinary scent marking. Using a large ($n = 33$), captive population, we collected serum samples to measure circulating sex steroids via enzyme immunoassay and urine samples to assay volatile chemicals via gas chromatography–mass spectrometry. Male binturongs had expectedly greater androgen concentrations than did females but, more unusually, had equal estrogen concentrations, which may be linked to male deference. Males also expressed a significantly richer array of volatile chemical compounds than did females. A subset of these volatile chemicals resisted decay at ambient temperatures, potentially indicating their importance as long-lasting semiochemicals. Among these compounds was 2-acetyl-1-pyrroline (2-AP), which is typically produced at high temperatures by the Maillard reaction and is likely to be responsible for the binturong's characteristic popcorn aroma. 2-AP, the only compound expressed by all of the subjects, was found in greater abundance in males than females and was significantly and positively related to circulating androstenedione concentrations in both sexes. This unusual compound may have a more significant role in mammalian semiochemistry than previously appreciated. Based on these novel data, we suggest that hormonal action and potentially complex chemical reactions mediate communication of the binturong's signature scent and convey information about sex and reproductive state.

Keywords Female dominance · Olfactory communication · Urinary signals · Reproductive endocrinology · 2-Acetyl-1-pyrroline · Viverrid

Communicated by: Sven Thatje

✉ Christine M. Drea
cdrea@duke.edu

[1] University Program in Ecology, Duke University, Durham, NC, USA

[2] Department of Evolutionary Anthropology, Duke University, Durham, NC, USA

[3] Department of Chemistry, Hendrix College, Conway, AR, USA

[4] Carnivore Preservation Trust (now Carolina Tiger Rescue), 1940 Hanks Chapel Road, Pittsboro, North Carolina 27312, USA

[5] Department of Biology, Duke University, Durham, NC, USA

[6] Present address: National Center for Environmental Health, Division of Laboratory Sciences, Clinical Chemistry Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

[7] Present address: Denver Zoo, 2300 Steele Street, Denver, Colorado 80205, USA

Introduction

Across mammalian taxa, urinary signaling is a well-conserved and critical mode of olfactory communication (Ralls 1971; Eisenberg and Kleiman 1972; Albone 1984), particularly prominent among members of the order Carnivora (Kleiman 1966; Ewer 1973; Anisko 1976; Macdonald 1980; Gorman and Trowbridge 1989). As exemplified by the male leg-lift behavior of canids (Berg 1944; Mertl-Millhollen et al. 1986) or spraying by felids (Brahmachary et al. 1992; Feldman 1994), the manner or frequency of urinary scent deposition can be highly sexually dimorphic, even if urine is a communicatory matrix used by both sexes. Various carnivorans, including large felids (*Acinonyx jubatus*: Burger et al. 2006;

Panthera leo: Andersen and Vulpius 1999), diverse canids (*Canis lupus*: Raymer et al. 1984; *Chrysocyon brachyurus*: Goodwin et al. 2013; *Lycaon pictus*: Apps et al. 2012; *Vulpes vulpes*: Jorgenson et al. 1978), mustelids (*Gulo gulo*: Wood et al. 2009; *Mustela furo*: Zhang et al. 2005), and ursids (*Ailuropoda melanoleuca*: Liu et al. 2013), express a rich array of volatile, urinary compounds that often differ by sex (Jorgenson et al. 1978; Raymer et al. 1984; Andersen and Vulpius 1999; Zhang et al. 2005; Burger et al. 2006; Liu et al. 2013). Both the patterning of signal deposition and of signal composition in these carnivoran species may serve a variety of inter- and intra-group functions, from delineating territories (Peters and Mech 1975; Sillero-Zubiri and Macdonald 1998), to advertising species, sex, individual identity, and reproductive state (Raymer et al. 1984; Zhang et al. 2005; Wood et al. 2009; Apps et al. 2012; Liu et al. 2013), to maintaining social hierarchies (Macdonald 1979; Asa et al. 1990; Sillero-Zubiri and Macdonald 1998) and promoting social cohesion (Rothman and Mech 1979; Porton 1983). Although androgen action typically underlies the sexually dimorphic patterns of urinary marking (Beach 1974; Hart 1974; Anisko 1976), in only a few carnivore studies have researchers examined the relationship between steroid hormones and urinary scent-signal composition (e.g., Raymer et al. 1984; Asa et al. 1990). Here, we report on the reproductive endocrine profiles and volatile urinary chemicals of captive, male and female binturongs (*Arctictis binturong*).

The binturong (also known as the bearcat) is a large, understudied viverrid, deriving from the dense forests of Southeast Asia (Pocock 1939; Grassman et al. 2005). It is unusual in that it possesses a semi-prehensile tail, has an omnivorous diet, and is reported to be female dominant (Wemmer and Murtaugh 1981; Abra 2010). The latter trait makes the binturong a compelling model for examining reproductive endocrinology, in both sexes. Most of the available literature on binturong social and reproductive behavior stems from studies of captive, breeding pairs and their offspring (Wemmer and Murtaugh 1981; Abra 2010). Presumed to be relatively solitary in the wild (Grassman et al. 2005), in captivity binturongs are normally housed alone or in mixed-sex dyads. When housed with males, females (that are heavier than males: Moresco and Larsen 2003) gain priority of access to food and sleeping sites (Wemmer and Murtaugh 1981; Abra 2010).

Binturongs of both sexes possess a perineal scent gland (associated with labial-like structures in the male: Story 1945) and adopt a stereotypic posture while depositing glandular secretions (Story 1945; Kleiman 1974). Both sexes also scent mark using urine, often soaking their tails and feet when voiding (Kleiman 1974), presumably to deposit critical urinary signals while ambulating. These shared morphological features and behavioral traits suggest a key role for scent marking in both sexes, as in other female-dominant species (Drea et al. 2002; Petty and Drea 2015). As is typical of most mammals (Ralls 1971), however, male binturongs engage in more scent-marking behavior than do females (Kleiman 1974). Nonetheless, female estrus leads to an upregulation of olfactory behavior in both sexes (Kleiman 1974). The prominent role of scent marking in female binturongs, coupled with the female's putative social dominance over males, motivates our search for hormonal mediation of olfactory signals in this species.

We used competitive enzyme immunoassays (EIA) to determine the concentrations of circulating sex steroids in adult binturongs. Specifically, we measured androstenedione (A_4), testosterone (T), and estradiol (E_2), in both sexes, because these steroids have been of interest to researchers studying other female-dominant mammals (reviewed in Drea 2009). In particular, A_4 is the common precursor of androgens and estrogens. Any unusual patterns in circulating concentrations of these steroids may be revelatory, both for comparative purposes and for better understanding potential routes to female dominance in binturongs.

We also used solid phase dynamic extraction (SPDE) coupled with gas chromatography–mass spectrometry (GC-MS) to identify the volatile components present in binturong urinary signals. We tested for sex differences, either in the expression of different compounds or in differing abundances of shared compounds. Curiously, binturongs, both in captivity and in nature (Wemmer and Murtaugh 1981; Grassman, personal communication), are reputed to smell of freshly cooked popcorn—an aroma that could stem from either glandular or urinary sources. In an earlier investigation of the chemical composition of glandular secretions, however, researchers detected several short-chain carboxylic acids (Weldon et al. 2000), none of which could be credited for the popcorn smell. We therefore explored a urinary source of this odor.

Lastly, we probed the potential hormonal mediation of odorant composition by asking if concentrations of our candidate steroids might be predictive of the abundance of major volatile compounds in binturong urine or of compounds that have putative semiochemical functions in other species. Such relationships might implicate an olfactory means of reproductive signaling.

Methods

Subjects and housing

Our subjects were 33 adult binturongs, including 13 reproductively intact females (mean age ± standard error [SE] 9.63 ± 1.0 years; range 4.5–17) and 20 males (mean age ± SE 8.55 ± 0.66 years; range 4.3–17). All of the males were hormonally intact, although five were vasectomized. We

confirmed that vasectomy had no influence on our results and therefore included all males in the study. Most (23 or 70 %) of the binturongs contributed both blood and urine samples (see below). The subjects were housed individually or in pairs in large enclosures (10 m × 20 m × 5 m) at the former Carnivore Preservation Trust (now the Carolina Tiger Rescue) in Pittsboro, North Carolina, USA. The subjects were fed a daily diet of local fruit, mice (*Mus musculus*), and kibble (Iams Chunks, Iams Company, Dayton, Ohio, USA), and water was freely available.

Sample collection and processing

Collection of the serum and urine samples was approved by the (then) Carnivore Preservation Trust's IACUC and occurred during routine yearly physical examinations. The timing of these examinations, conducted between November and December 2001, minimized any potential seasonal influences. The breeding season of binturongs in the wild is unknown; in captivity, binturongs can breed year round, but have seasonal birth peaks (Wemmer and Murtaugh 1981). For sample collection, subjects were anesthetized using a combination of ketamine (Ketaset®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA), medetomidine (Domitor®, Pfizer Animal Health, Exton, Pennsylvania, USA), and butorphanol (Torbugesic®, Fort Dodge Animal Health), following published procedures (Moresco and Larsen 2003).

We collected blood samples from 29 of the subjects (9 females, 20 males). We drew blood from the jugular vein with a 20-ga, butterfly catheter and transferred the samples to 3-mL serum separator tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, New Jersey, USA). We stored the samples of decanted serum at Duke University (Durham, North Carolina, USA) at −80 °C, until analysis.

We collected urine samples from 26 of the subjects (9 females, 17 males) by putting gentle, but firm, pressure on the bladder (see delBarco-Trillo et al. 2013). We stored the urine samples at Duke University, at −80 °C, until shipment to Hendrix College (Conway, Arkansas, USA) for analysis. We shipped these samples overnight, on dry ice, in two batches. The shipped samples from batch 1 ($n = 19$: 6 F, 13 M) were kept frozen during transit, whereas the shipped samples from batch 2 ($n = 7$: 3 F, 4 M) were delayed in transit by 12 h and arrived thawed. Upon arrival, the samples from both shipments were immediately stored at −70 °C until analysis.

Hormone assays

We determined serum concentrations of A_4, T, and E_2 using commercial, competitive EIA kits (ALPCO Diagnostics, Salem, New Hampshire, USA). We validated all the EIA assays for (1) analyte recovery, by spiking pooled serum samples with a range of known analyte values (A_4 0.05–5.0 ng/mL; T 0.04–2.5 ng/mL; E_2 10–500 pg/mL) representing the low, middle, and high regions of each standard curve, and comparing the observed and expected results, and (2) linearity, by running a serial dilution of the pooled serum and comparing the slopes against the standard curves. For all assays, recovery ranged from 83.5 to 105.5 % and each dilution curve was linear and parallel to the appropriate assay standard curve. The A_4 assay has a sensitivity of 0.04 ng/mL using a 25-μL dose, with an intra- and inter-assay coefficient of variation (CV) of 5.23 and 8.7 %, respectively. The T assay has a sensitivity of 0.02 ng/mL using a 50-μL dose, with an intra- and inter-assay CV of 7.9 and 7.3 %, respectively. The E_2 assay has a sensitivity of 10 pg/mL using a 50-μL dose, with an intra- and inter-assay CV of 7.7 and 8.7 %, respectively. We performed all assays in duplicate. If a sample's duplicate CV exceeded the intra-assay CV, it was subsequently re-run on a different plate. Samples from three individuals had circulating A_4 values beyond the range of detection, and samples from two individuals had E_2 values that were similarly out of range. These subjects were therefore dropped, respectively, from the A_4 and E_2 analyses.

Analysis of urinary volatiles

We determined the volatile compounds in binturong urine using SPDE/GC-MS procedures. We sealed a 0.5-mL aliquot of each urine sample, combined with 300 mg of reagent grade (>99 % pure) NaCl (American Chemical Society, Washington, DC, USA; to force volatile organic compounds out of solution) and a small Teflon®-coated stir bar, in a 20-mL screw-top vial with a threaded, metallic septum cap (silicone/polytetrafluoroethylene-layered septum; www.chromtech.de, or www.bgb-analytik.com). We programmed multiple samples to run automatically, using the Combi PAL robot and associated SPDE hardware and software (www.chromtech.de). The SPDE needle was internally coated with activated charcoal (Carboxen®)–polydimethylsiloxane. After incubating the stirred sample at 37 °C for 15 min, the headspace was sampled for 13 min (200 up-and-down 1-mL strokes of the syringe). Desorption of adsorbed analytes was at 250 °C in the GC inlet. We conducted GC-MS analyses using an Agilent 6890N GC and 5973N Mass Selective Detector (Agilent Technologies, Santa Clara, California, USA). The capillary GC column was an Equity 1 (bonded; polydimethylsiloxane), 60 m × 0.32 mm ID, 1 μm film thickness (Supelco cat. no. 28058U, Bellefonte, Pennsylvania, USA). The GC oven was temperature programmed to hold for 2 min at 35 °C, followed by ramping to 180 °C at 3.75 °C/min, where it was held for 5 min before ramping at 20 °C/min, to a final temperature of 250 °C, where it was held for 10 min. The mass

spectrometer was programmed at 3.09 scans/s for a mass scan of 30–500 amu.

The identities of all compounds reported herein from SPDE/GC-MS were verified by GC retention times and mass spectra when compared to commercial samples (from Sigma-Aldrich or Fisher Scientific) or synthesized samples, as well as by comparison to the NIST mass spectral library (version 2002). In particular, one of these compounds, 2-acetyl-1-pyrroline (2-AP, see Results), was prepared by the procedure of Buttery et al. (1983). For quantitative comparisons of the volatile compounds, we used peak areas from the total ion chromatogram (TIC) profiles to reflect relative abundances.

Analysis of dietary volatiles

Although identified in small concentrations in mouse urine (Kwak et al. 2008, 2013) and in certain exotic fruits (Wong et al. 1992; Hayata et al. 2003), 2-AP is typically associated with prepared foods and likely synthesized via the Maillard reaction (Adams and De Kimpe 2006) at supraphysiological temperatures. We therefore analyzed the headspace of both wet and dry kibble to test if the Iams Chunks kibble fed to our subjects might have influenced the volatile urinary compounds of binturongs. We used the same SPDE/GC-MS analytical approach as described above, except that we neither added NaCl nor a stir bar. We used approximately 0.60 g of Iams chunks; for the wet runs, we also added 2 mL of deionized water to the sample vial.

Statistical analyses

We tested for sex differences in circulating concentrations of A_4, T, and E_2. Because the hormonal data were non-normally distributed, we used two-tailed Wilcoxon Rank Sum tests performed in JMP Pro (version 12.0).

For our chemical analyses, we first investigated if the GC-MS profiles of the urine samples that underwent the extra thaw during shipping (i.e., batch 2) were equivalent to those that remained frozen prior to analysis (i.e., batch 1). We tested for potential differences in chemical richness (the number of chemical compounds calculated for each sample: McCune et al. 2002) using Student's t test in JMP Pro (version 12.0). We then investigated if the compounds ($n = 11$) that were present in both sample batches differed in their relative abundances. Following established protocols (Drea et al. 2013), we reduced the dimensionality of this dataset by calculating principal components (PCs). We retained those PCs with eigenvalues >1 and that explained >1 % of the variation. We used these PCs as covariates in a linear discriminant (LD) analysis, in which we entered sample batch as the X category. We used Wilks' λ test of group differences to test for significance. We likewise conducted PC and LD analyses in JMP Pro (version 12.0).

Using the same types of analyses, we next tested for sex differences in the volatile chemicals present in binturong urine. We tested for differences in chemical richness using Student's t test; however, because the extra thaw experienced by batch 2 significantly influenced chemical richness (see below), we only considered the samples from batch 1 in this analysis. We then examined if a relationship existed between sex and the relative abundance of compounds expressed equally in both sample batches. Specifically, we used the PCs calculated for the above analysis as the covariates in a second LD analysis, for which we entered sex as the X category. We likewise used Wilks' λ test of group differences to test for significance. For those individuals whose samples remained frozen prior to analysis (i.e., batch 1), we additionally tested for relationships between A_4, T, and E_2 values and chemical richness, using linear regression in GraphPad Prism version 5.04. Lastly, we tested for relationships between steroid concentrations and specific chemical compounds that were likely to provide a semiochemical function. Specifically, we determined if the sexes expressed certain key compounds in similar abundances, using a Wilcoxon Rank Sum test, and examined the relationship between compound abundance and A_4, T, and E_2 concentrations, using linear regression in JMP Pro (version 12.0).

Results

Sex differences in reproductive hormones

Compared to females, male binturongs had significantly greater concentrations of circulating A_4 (Wilcoxon Rank Sum test: $Z = 3.56$, $p < 0.001$; Fig. 1a) and T (Wilcoxon Rank Sum test: $Z = 3.54$, $p < 0.001$; Fig. 1b). In contrast, male and female binturongs had similar concentrations of circulating E_2 (Wilcoxon Rank Sum test: $Z = 0.08$, $p > 0.9$; Fig. 1c).

Volatile chemicals in binturong urine

The binturongs in our population expressed 29 volatile urinary compounds, representing a mixture of acids, alcohols, aldehydes, hydrocarbons, ketones, and 2-AP (Table 1). The latter compound was the only one expressed by every subject (Fig. 2). Of all the compounds, 16 (55 %) were male specific, whereas none were female specific (Fig. 2).

Volatile chemicals in captive binturong diet

Overall, we found that the volatile chemical profiles of kibble samples differed dramatically from those of the urinary samples (data not shown). Only the following four components were common to both the binturong and its diet, but in relatively low abundance in the diet headspace: acetic acid, 2-

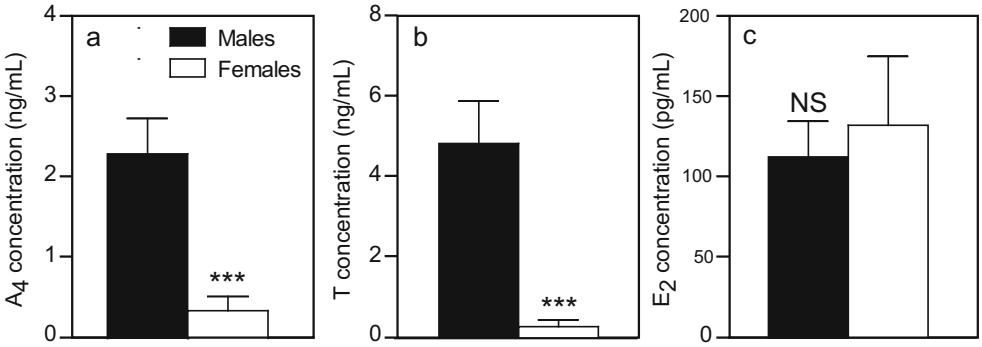

Fig. 1 Sex differences in binturongs in mean + SE serum concentrations of reproductive hormones, including **a** androstenedione (A_4), **b** testosterone (T), and **c** estradiol (E_2). ***Denotes $p < 0.001$; *NS* denotes non-significance

heptanone, benzaldehyde, and nonanal (see Table 1). Most notably, we found no evidence of 2-AP in the Iams Chunks kibble.

The influence of ambient temperature on volatile urinary compounds

As would be expected (Drea et al. 2013), the samples that were kept frozen prior to analysis were significantly richer in chemical compounds than those that experienced an extra thaw at ambient temperatures (*t* test: $t_{23.99} = 5.8134$, $p < 0.001$; Fig. 3a). Nevertheless, the compounds that were present in both batches were present in similar relative abundances. More specifically, from the compounds that were present in both sample batches ($n = 11$), we extracted five PCs that cumulatively explained 82.9 % of the variation. We could detect no effect of sample batch on these PCs: LD analysis misclassified nine (35 %) samples (or individuals) into the wrong sample batch (one vs. two) and revealed no significant difference between samples that were either kept frozen or had experienced an extra thaw prior to analysis (Wilks' $\lambda = 0.824$, $p = 0.5298$; Fig. 3b).

Sex-related patterns in volatile urinary compounds

Male binturongs expressed a significantly richer array of volatile compounds in their urine than did their female counterparts (*t* test: $t_{15.77} = 4.5997$, $p < 0.001$; Figs. 2 and 4a). 2-AP, specifically, was expressed in greater relative abundance by male than by female binturongs (Wilcoxon Rank Sum test: $Z = 2.48$, $p < 0.02$; Figs. 2 and 4b). Lastly, of the compounds that reliably appeared in both batches, LD analysis revealed a significant difference between the sexes (Wilks' $\lambda = 0.369$, $p < 0.001$; Fig. 4c) and classified all but one (96.2 %) sample (or individual) into the correct sex category.

Endocrine-volatile chemical relationships

We could detect no relationship between any of the steroids (A_4, T, or E_2) and chemical richness ($p > 0.05$ for all; data not shown). Because 2-AP was expressed by all of the subjects and, therefore, may be an important semiochemical for binturongs, we tested for relationships between steroid concentrations and this particular compound: There was no relation between 2-AP and either T ($R^2 = 0.001$, $df = 21$, $p = 0.9092$, data not shown) or E_2 ($R^2 = 0.031$, $df = 19$, $p = 0.4466$, data not shown), but 2-AP was positively and significantly related to A_4 concentrations ($R^2 = 0.47$, $df = 18$, $p < 0.001$; Fig. 5).

Discussion

To better understand female dominance in the binturong, we examined adult sex steroids in relation to volatile urinary chemicals; through these efforts, we provide novel information about this species' reproductive physiology and chemical communication. Our endocrine findings lead us to suspect that the display of female dominance during the intersexual interactions of binturongs may owe to female size advantage, coupled potentially with estrogen-mediated male deference. Our chemical analyses contribute to identifying 2-AP as a putative semiochemical—one that explains the binturong's popcorn aroma—and also point to a significant role for volatile urinary compounds, possibly under hormonal mediation, in the communication of species identity, individual sex, and reproductive state.

Across vertebrate taxa, males and females typically have greater circulating concentrations of androgens and estrogens, respectively (Drea 2009). Nonetheless, in some female-dominant species, androgen concentrations in females can be raised (Drea 2007; Petty and Drea 2015), potentially reflecting physiological "masculinization" (Drea 2009), such that the hormonal sex difference is reduced, absent, or even reversed (Glickman et al. 1992; Koren et al. 2006; Davies et al. unpublished data). Yet, for the female-dominant binturong, the reduction in sex difference was observed, not in androgens, but in estrogens. Although binturongs evidenced the typical mammalian sex differences in androgens, with males having greater abundances of A_4 and T than females, we could find no discernible sex difference in circulating E_2 concentrations. That male binturongs showed relatively high

Table 1 Chemical compounds detected in the headspace of binturong urine

Retention time (min)	Tentative compound Identification	Presence after time delay	% of animals expressing compound[a]	Sex(es) expressing compound
5.75	Acetone	Yes	34.6 (9/26)	M, F
6	Isopropyl alcohol	No	31.6 (6/19)	M, F
8.29	2-Butanone	Yes	42.3 (11/26)	M, F
9.05	Ethyl acetate	No	15.8 (3/19)	M
9.6	Acetic acid	No	68.4 (13/19)	M, F
11.74	2-Pentanone	Yes	73.1 (19/26)	M, F
12.19	3-Pentanone	Yes	30.8 (8/26)	M
13.2	Heptane	No	26.3 (5/19)	M
13.9	3-Buten-1-ol, 3-methyl-	Yes	11.5 (3/26)	M
15.99	1,3-Butadiene, 2-methyl-	Yes	19.2 (5/26)	M
16.35	3-Hexanone	No	31.6 (6/19)	M
18.94	3-Hexanone, 5-methyl-	No	52.6 (10/19)	M
20.73	4-Heptanone	Yes	61.5 (16/26)	M, F
21.5	2-Heptanone	No	31.6 (6/19)	M, F
22.78	2-Acetyl-1-pyrroline	Yes	100 (26/26)	M, F
23.9	Pentanoic acid, 2-methyl-	No	26.3 (5/19)	M
24.15	Unknown 1	Yes	46.1 (12/26)	M, F
24.75	Benzaldehyde	Yes	38.5 (10/26)	M
28.62	Benzene, 1-methyl-2/4-(1-methylethyl)-	No	15.8 (3/19)	M
28.6	Ethanone, 1-(1H-pyrrol-2-yl)-	No	36.8 (7/19)	M
29.03	Limonene	No	31.6 (6/19)	M, F
29.5	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	No	10.5 (2/19)	M
31.34	Benzene, 1-methyl-4-(1-methylethenyl)-	No	10.5 (2/19)	M
31.52	Unknown 2	No	36.8 (7/19)	M, F
31.53	Nonanal	No	31.6 (6/19)	M, F
33.17	1-Pentene, 2-methyl-	No	15.8 (3/19)	M
35.47	Unknown 3	No	15.8 (3/19)	M
35.82	Decanal	Yes	73.1 (19/26)	M, F
37.75	3-Carene	No	36.8 (7/19)	M

[a] Compounds that were detected in both sample batches are presented for all individuals (denominator $n = 26$), whereas compounds that were not detected after a time delay are presented only for the individuals analyzed in batch 1 (denominator $n = 19$)

concentrations of circulating E_2 perhaps implicates male deference, more so than androgen-mediated female aggression, in this species' intersexual interactions.

Because estrogens have been studied primarily in the context of female reproduction, their role in mediating adult male behavior remains underappreciated. When estrogens in adult males have been examined, they often have been linked to declining reproductive function, including decreased sperm counts and unsuccessful sexual behavior (Toppari et al. 1996; Bjerselius et al. 2001), or to increased paternal care (Wynne-Edwards 2001). Nonetheless, the males of some species (*Equus ferus*: Raeside 1979; Bono et al. 1982; *Rangifer tarandus*: Bubenik et al. 1997), including various female-dominant lemurs (*Lemur* and *Eulemur* spp.: Petty 2015), maintain normal reproductive function while also expressing raised estrogen concentrations. Ultimately, a deeper understanding of behavioral and reproductive endocrinology, including female dominance (and perhaps the male's unusual genital anatomy), will require continued comparative research into the function of "heterologous hormones" in both sexes, specifically of androgens in females and estrogens in males.

With regard to chemical signaling in the binturong, both sexes possess perineal scent glands (Pocock 1915; Story 1945), the secretions of which predominantly contain carboxylic acids (Weldon et al. 2000) that neither explain the binturong's distinctive popcorn aroma nor encode the signaler's sex. By contrast, binturong urine contained a richer array of volatiles that likely encode reproductively relevant information. Notably, as the only compound expressed by all binturongs, 2-AP may serve as a species identifier.

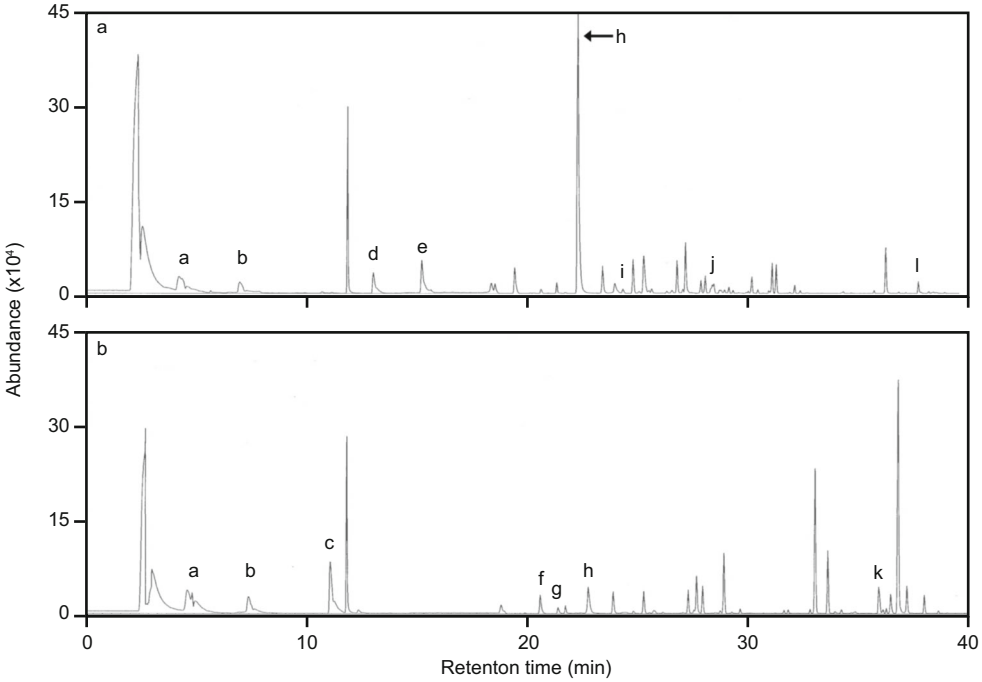

Fig. 2 Representative gas chromatograms showing the volatile urinary compounds present in a **a** male and **b** female binturong. *Letters* identify some of the major volatile compounds that are either shared by both sexes, expressed in different relative abundances, or present in the male only: *a* acetone, *b* 2-butanone, *c* 2-pentanone, *d* 3-buten-1-ol, 3-methyl-, *e* 1,3-butadiene, 2-methyl-, *f* 4-heptanone, *g* 2-heptanone, *h* 2-AP (2-acetyl-1-pyrroline), *i* benzaldehyde, *j* ethanone, 1-(1H-pyrrol-2-yl)-, *k* decanal, and *l* 3-carene

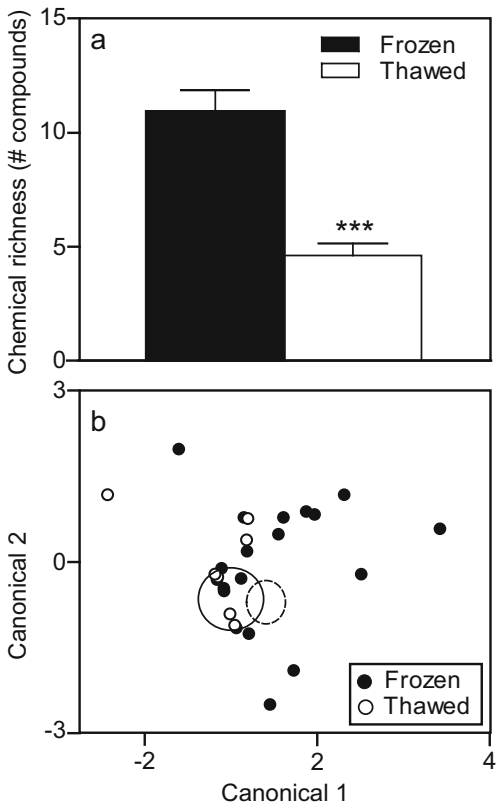

Fig. 3 Effects of sample thaw on the volatile chemical compounds in binturong urine. Depicted are **a** the differences in mean + SE chemical richness (or the number of components detected) and **b** a linear discriminant (LD) plot of the principal components calculated from the relative abundance of compounds present in both sample types (thawed and frozen). ***Denotes $p < 0.001$

Because it is partly responsible for the characteristic smell of popped corn (Schieberle 1991) and aromatic rice (e.g., jasmine or basmati: Buttery et al. 1982, 1983; Yoshihashi 2002), 2-AP likely explains the binturong's signature scent. In only one other carnivore, the tiger (*P. tigris*), has 2-AP been suggested to be a putative semiochemical (Brahmachary et al. 1990; Brahmachary 1996), although there are anecdotal reports of other carnivores producing a similar, but less intense, aroma (particularly from their feet). Given that the synthesis of 2-AP has been thoroughly investigated in the context of food preparation (Bradbury et al. 2008), it is important to note that we detected no 2-AP in the binturongs' commercially prepared diet. It also occurs in only small amounts in mouse urine (Kwak et al. 2008, 2013). The diet provided to binturongs in captivity could potentially contribute to four other urinary compounds we detected, including acetic acid, 2-heptanone, benzaldehyde, and nonanal; however, these are common components of carnivore urine and are therefore likely to also be endogenously derived (Andersen and Vulpius 1999; Zhang et al. 2005; Burger et al. 2006; Wood et al. 2009).

Male binturongs had a greater number of volatile compounds in their urine than did females, and many of these compounds, including 2-AP, also occurred in greater abundance in males than in females. Such sex differences are consistent across a broad range of vertebrate taxa (Müller-Schwarze 2006; Drea 2015). Likewise, several of the male-specific compounds reported herein are sex specific in other species (see, for example, Table 3 in delBarco-Trillo et al. 2011). These traditional male biases in compound richness and abundance, coupled with the strong sex specificity of certain compounds, suggest that binturong urinary cues

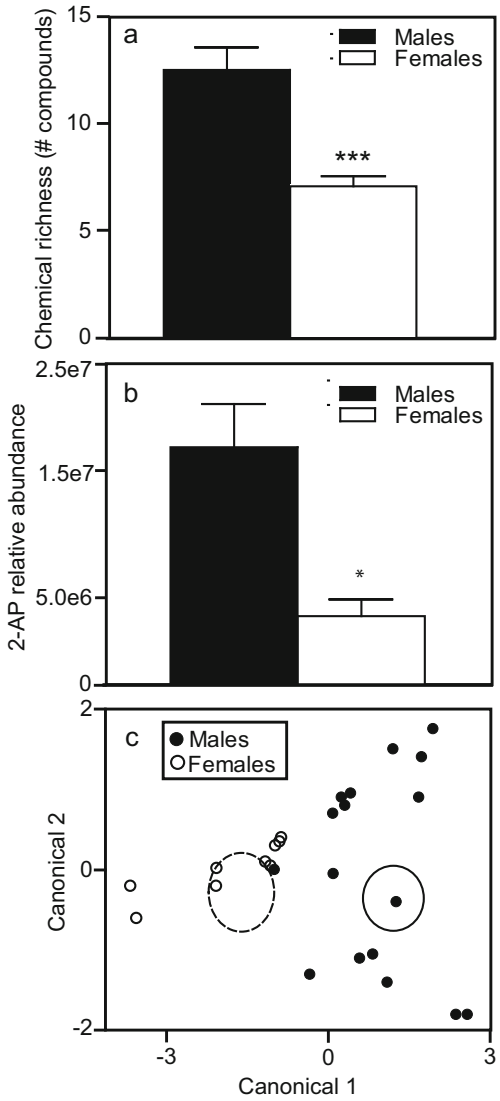

Fig. 4 Sex differences in the chemical compounds detected in binturong urine. Depicted are **a** mean + SE chemical richness (or the number of components detected), **b** mean + SE relative abundance of 2-acetyl-1-pyrroline (2-AP), and **c** a linear discriminant (LD) plot of the principal components calculated for the subset of compounds present across sample batches (i.e., frozen vs. thawed). *Denotes $p < 0.05$; ***denotes $p < 0.001$

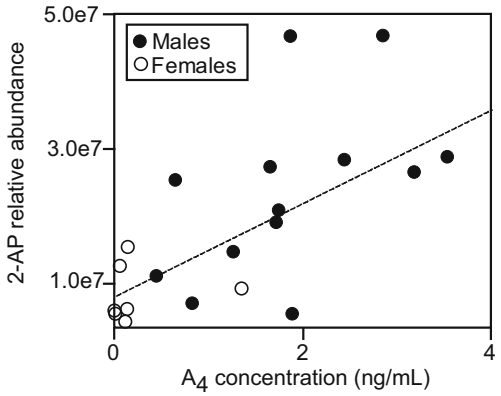

Fig. 5 Relationship, across both sexes, between the relative abundances of 2-acetyl-1-pyrroline (2-AP) in binturong urine and the concentrations of androstenedione (A_4) in binturong serum

provide redundant information about the signaler's sex. Nevertheless, the direction of these biases contrast with patterns evident in the scent secretions of other female-dominant species, in which female chemical richness can exceed that of males (Scordato et al. 2007; Boulet et al. 2009; delBarco-Trillo et al. 2012). Thus, as with their endocrine patterns, binturongs also differ from other female-dominant species in terms of their sex differences in the chemical composition of their scent signatures.

Lastly, in the context of reproductive function, the positive relation across sexes between 2-AP and the prohormone A_4 is consistent with hormonal mediation of binturong scent signals. Because A_4 is the immediate precursor in the biosynthesis of both testosterone and estrone, there also may be a role for urinary signaling of reproductive state in both male and female binturongs.

Chemical communication has been considered to be particularly relevant for solitary species because, unlike visual and vocal signals, olfactory signals are long lasting and can convey information in the absence of the signaler (Alberts 1992). Beyond safely signaling territorial boundaries (i.e., without the need for social interaction or confrontation), enduring chemical cues may be critical for advertising sex or reproductive state in solitary species. We nevertheless have limited appreciation for the tenacity of specific compounds or blends of compounds under natural environmental conditions because the chemical decay of odorants has been examined in only a few studies. In mice, for instance, proteins excreted in urine function to prolong signal availability (Hurst et al. 1998). In bull elephants (*Loxodonta africana* and *Elephas maximus*) undergoing musth, certain alcohols and ketones can increase in concentration as olfactory signals age (Goodwin et al. 2012). That certain compounds may be increasingly detectable after samples have been exposed, over time, to ambient temperatures may implicate bacterial regulation of chemical information (Alberts 1992; Archie and Theis 2011; Goodwin et al. 2012; Ezenwa and Williams 2014). The age of an odorant can also influence how signal receivers perceive information. In ring-tailed lemurs (*Lemur catta*), for example, males preferentially sniff fresh odorants, but preferentially lick decayed odorants, potentially indicating their respective assessment of ephemeral, volatile components versus long-lasting, non-volatile components (Greene et al. 2016).

Although ultra-rapid and portable GC analyzers (e.g., the zNose®) offer the possibility of measuring chemical change in natural environments (Staples and Viswanathan 2005), studying odorant decay or persistence can present logistical challenges, including if freezing differentially affects certain compounds (Hoffmann et al. 2009) or interrupts bacterial fermentation. We used the difference in shipping

duration to serendipitously examine the effects of decay on the volatile composition of binturong urine. Fewer than half of the original compounds remained in equal relative abundances after samples underwent a thaw. Whereas the "lost" components may have signaled relevant information immediately after deposition, those components that persisted might be considered candidate semiochemicals in long-term communication (i.e., in the absence of the signaler: Alberts 1992).

Several key compounds persisted in "decayed" binturong urine, including 2-AP. Although somewhat unstable in isolation, 2-AP is relatively stable in dilute liquids (Buttery et al. 1983) such as urine (Kwak et al. 2013); however, given (a) the high temperatures needed to synthesize 2-AP via the Maillard reaction, (b) the minimal likelihood that 2-AP at the concentrations we observed derived from the few mice in the binturong's diet, and (c) the absence of 2-AP from the binturong's commercial diet, we lack an understanding of how binturongs (or other mammals) produce this compound. One possibility is that symbiotic bacteria produce 2-AP during normal fermentation processes in the gut. Indeed, *Bacillus cereus* has been shown to synthesize 2-AP without a Maillard reaction (Romanczyk et al. 1995; Adams and De Kimpe 2007) and is a common microbe found in vertebrate intestinal systems (Swiecicka 2008). In future studies, it would be interesting to determine if this bacterium inhabits the gastro-intestinal tracts of binturongs or other carnivores and if it can readily synthesize 2-AP. 2-AP's longevity, post urinary deposition, might be further explained by slow-release carrier proteins or continued microbial synthesis.

Future behavioral studies could help resolve if female dominance in binturongs is mediated by female aggression or, as is possibly suggested by adult male endocrine profiles, by male deference. Indeed, for the binturong and most other species, the role of heterologous hormones in regulating sociality and reproduction remains poorly understood. Research programs focused on the role of androgens in females have revealed insights about female masculinization (Glickman et al. 1992; Drea 2009; Petty and Drea 2015; Davies et al. unpublished data); however, the role of estrogens in regulating normal male behavior also may be relevant. Our study of an underrepresented species has led to identifying 2-AP as a putative semiochemical, one that has rarely been attributed a role in mammalian communication (Brahmachary 1996), but that may be more widespread in carnivores than previously recognized. This unusual compound highlights the fact that we lack understanding of the processes by which many of the chemicals that function in mammalian olfactory communication are produced, namely, whether they are synthesized by mammalian tissues or produced by symbiotic bacteria as part of routine metabolism. Ultimately, linking chemical communication to reproductive function in unusual species will contribute to a broader understanding of social evolution.

Acknowledgments We would like to thank the Carnivore Preservation Trust (now the Carolina Tiger Rescue), in particular Kathryn Bertok, for their collaboration in this project and for the excellent care provided to the animals. We thank Joseph Petty and Jillian Wisse for their technical assistance with validating the endocrine assays. The assistance of Qin Yin and Innocent H. Harelimana with the GC-MS data analysis is greatly appreciated. Funds were provided by an Undergraduate Research Support grant from Duke University (to LKG) and by National Science Foundation grant, IOS-1021633 (to CMD). Financial support at Hendrix College was provided via a Distinguished Professor grant, the Odyssey Program, and John and Laura Byrd (to TEG).

Compliance with ethical standards

Human and animal rights and informed consent The animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

References

Abra L (2010) Notes on the development and behaviour of two binturong *Arctictus binturong* litters born at Taronga Zoo, Sydney. Int Zool Yearb 44:238–245. doi:10.1111/j.1748-1090.2009.00102.x

Adams A, De Kimpe N (2006) Chemistry of 2-acetyl-1-pyrroline, 6-acetyl-1, 2, 3, 4-tetrahydropyridine, 2-acetyl-2-thiazoline, and 5-acetyl-2,3-dihydro-4 H-thiazine: extraordinary Maillard flavor compounds. Chem Rev 106:2299–2319. doi:10.1021/cr040097y

Adams A, De Kimpe N (2007) Formation of pyrazines and 2-acetyl-1-pyrroline by *Bacillus cereus*. Food Chem 101:1230–1238. doi:10.1016/j.foodchem.2006.03.027

Alberts AC (1992) Constraints on the design of chemical communication systems in terrestrial vertebrates. Am Nat: S62-S89

Albone ES (1984) Mammalian semiochemistry: the investigation of chemical signals between mammals. Wiley, New York

Andersen K, Vulpius T (1999) Urinary volatile constituents of the lion, *Panthera leo*. Chem Senses 24:179–189. doi:10.1093/chemse/24.2.179

Anisko JJ (1976) Communication by chemical signals in Canidae. In: Doty R (ed) Mammalian olfaction, reproductive processes, and behavior. Academic, New York, pp 283–293

Apps P, Mmualefe L, McNutt JW (2012) Identification of volatiles from the secretions and excretions of African wild dogs (*Lycaon pictus*). J Chem Ecol 38:1450–1461. doi:10.1007/s10886-012-0206-7

Archie EA, Theis KR (2011) Animal behaviour meets microbial ecology. Anim Behav 82:425–436. doi:10.1016/j.anbehav.2011.05.029

Asa CS, Mech LD, Seal US, Plotka ED (1990) The influence of social and endocrine factors on urine-marking by captive wolves (*Canis lupus*). Horm Behav 24:497–509. doi:10.1016/0018-506X(90)90038-Y

Beach FA (1974) Effects of gonadal hormones on urinary behavior in dogs. Physiol Behav 12:1005–1013. doi:10.1016/0031-9384(74)90148-6

Berg IA (1944) Development of behavior: the micturition pattern in the dog. J Exp Psychol 34:343–368. doi:10.1037/h0055459

Bjerselius R, Lundstedt-Enkel K, Olsén H, Mayer I, Dimberg K (2001) Male goldfish reproductive behaviour and physiology are severely affected by exogenous exposure to 17β-estradiol. Aquat Toxicol 53:139–152. doi:10.1016/S0166-445X(00)00160-0

Bono G, Minori D, Gaiani R, Mattioli M (1982) Concentrations of oestrone sulphate, androgens and LH in the peripheral plasma of

mating stallions. J Reprod Fertil 66:17–22. doi:10.1530/jrf.0.0660017

Boulet M, Charpentier MJE, Drea CM (2009) Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in a primate. BMC Evol Biol 9:281. doi:10.1186/1471-2148-9-281

Bradbury LM, Gillies SA, Brushett DJ, Waters DL, Henry RJ (2008) Inactivation of an aminoaldehyde dehydrogenase is responsible for fragrance in rice. Plant Mol Biol 68:439–449. doi:10.1007/s11103-008-9381-x

Brahmachary R (1996) The expanding world of 2-acetyl-1-pyrroline. Curr Sci 71:257–258

Brahmachary RL, Sarkar MP, Dutta J (1990) The aroma of rice … and tiger. Nature 344:26. doi:10.1038/344026b0

Brahmachary RL, Sarkar MP, Dutta J (1992) Chemical signals in the tiger. Chem Signals in Vertebrates 6, Springer: 471–475. doi: 10.1007/978-1-4757-9655-1_72

Bubenik GA, Schams D, White RJ, Rowell J, Blake J, Bartos L (1997) Seasonal levels of reproductive hormones and their relationship to the antler cycle of male and female reindeer (*Rangifer tarandus*). Comp Biochem Physiol Part B: Biochem Mol Biol 116:269–277. doi:10.1016/S0305-0491(97)00183-1

Burger BV, Visser R, Moses A, Le Roux M (2006) Elemental sulfur identified in urine of cheetah, *Acinonyx jubatus*. J Chem Ecol 32:1347–1352. doi:10.1007/s10886-006-9056-5

Buttery RG, Ling LC, Juliano BO (1982) 2-Acetyl-1-pyrroline: an important aroma component of cooked rice. Chem Indust: 958–959

Buttery RG, Ling LC, Juliano BO, Turnbaugh JG (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agric Food Chem 31:823–826

delBarco-Trilllo J, Sacha CR, Dubay GR, Drea CM (2012) *Eulemur*, me lemur: the evolution of scent-signal complexity in a primate clade. Phil Trans R Soc B: Biol Sci 367:1909–1922. doi:10.1098/rstb.2011.0225

delBarco-Trillo J, Harelimana IH, Goodwin TE, Drea CM (2013) Chemical differences between voided and bladder urine in the aye-aye (*Daubentonia madagascariensis*): implications for olfactory communication studies. Am J Primatol 75:695–702. doi:10.1002/ajp.22083

delBarco-Trillo J, Burkert BA, Goodwin TE, Drea CM (2011) Night and day: the comparative study of strepsirrhine primates reveals socioecological and phylogenetic patterns in olfactory signals. J Evol Biol 24:82–98. doi:10.1111/j.1420-9101.2010.02145.x

Drea CM (2007) Sex and seasonal differences in aggression and steroid secretion in *Lemur catta*: are socially dominant females hormonally 'masculinized'? Horm Behav 51:555–567. doi:10.1016/j.yhbeh.2007.02.006

Drea CM (2009) Endocrine mediators of masculinization in female mammals. Curr Dir Psychol Sci 18:221–226. doi:10.1111/j.1467-8721.2009.01640.x

Drea CM (2015) D'scent of man: a comparative survey of primate chemosignaling in relation to sex. Horm Behav 68:117–133. doi:10.1016/j.yhbeh.2014.08.001

Drea CM, Vignieri SN, Cunningham SB, Glickman SE (2002) Responses to olfactory stimuli in spotted hyenas (*Crocuta crocuta*): I. Investigation of environmental odors and the function of rolling. J Comp Psychol 116:331–341. doi:10.1037//0735-7036.116.4.331

Drea CM, Boulet M, delBarco-Trillo J, Greene LK, Sacha CR, Goodwin TE, Dubay GR (2013) The "secret" in secretions: methodological considerations in deciphering primate olfactory communication. Am J Primatol 75:621–642. doi:10.1002/ajp.22143

Eisenberg JF, Kleiman DG (1972) Olfactory communication in mammals. Ann Rev Ecol Syst 3:1–32. doi:10.1146/annurev.es.03.110172.000245

Ewer RF (1973) The carnivores. Cornell University Press, Ithaca

Ezenwa VO, Williams AE (2014) Microbes and animal olfactory communication: where do we go from here? BioEssays 36:847–854. doi:10.1002/bies.201400016

Feldman HN (1994) Methods of scent marking in the domestic cat. Can J Zool 72:1093–1099. doi:10.1139/z94-147

Glickman S, Frank L, Pavgi S, Licht P (1992) Hormonal correlates of 'masculinization' in female spotted hyaenas (*Crocuta crocuta*). 1. Infancy to sexual maturity. J Reprod Fertil 95:451–462. doi:10.1530/jrf.0.0950451

Goodwin TE, Broederdorf LJ, Burkert BA, Hirwa IH, Mark DB, Waldrip ZJ, Kopper RA, Sutherland MV, Freeman EW, Hollister-Smith JA (2012) Chemical signals of elephant musth: temporal aspects of microbially-mediated modifications. J Chem Ecol 38:81–87. doi:10.1007/s10886-011-0056-8

Goodwin TE, Songsasen N, Broederdorf LJ, Burkert BA, Chen CJ, Jackson SR, Keplinger KB, Rountree ME, Waldrip ZJ, Weddell ME (2013) Hemiterpenoids and pyrazines in the odoriferous urine of the maned wolf (*Chrysocyon brachyurus*). In East ML & Dehnhard D (eds) Chemical Signals in Vertebrates 12, Springer, New York, pp 171–184. doi: 10.1007/978-1-4614-5927-9_13

Gorman ML, Trowbridge BJ (1989) The role of odor in the social lives of carnivores. In: Gittleman JL (ed) Carnivore Behavior, Ecology, and Evolution, Springer: 57–88. doi: 10.1007/978-1-4757-4716-4_3

Grassman LI, Tewes ME, Silvy NJ (2005) Ranging, habitat use and activity patterns of binturong *Arctictis binturong* and yellow-throated marten *Martes flavigula* in north-central Thailand. Wildl Biol 11:49–57. doi:10.2981/0909-6396(2005)11[49:RHUAAP]2.0.CO;2

Greene LK, Grogan KE, Smyth KN, Adams CA, Klager SA, Drea CM (2016) Mix it and fix it: functions of composite olfactory signals in ring-tailed lemurs. R Soc Open Sci 3:160076. doi:10.1098/rsos.160076

Hart BL (1974) Gonadal androgen and sociosexual behavior of male mammals: a comparative analysis. Psychol Bull 81:383. doi:10.1037/h0036568

Hayata Y, Sakamoto T, Maneerat C, Li X, Kozuka H, Sakamoto K (2003) Evaluation of aroma compounds contributing to muskmelon flavor in porapak Q extracts by armona extract dilution analysis. J Agric Food Chem 51:3415–3418. doi:10.1021/jf0209950

Hoffmann F, Musolf K, Penn DJ (2009) Freezing urine reduces its efficacy for eliciting ultrasonic vocalizations from male mice. Physiol Behav 96:602–605. doi:10.1016/j.physbeh.2008.12.014

Hurst JL, Robertson DHL, Tolladay U, Beynon RJ (1998) Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. Anim Behav 55:1289–1297. doi:10.1006/anbe.1997.0650

Jorgenson J, Novotny M, Carmack M, Copland G, Wilson S, Katona S, Whitten W (1978) Chemical scent constituents in the urine of the red fox (*Vulpes vulpes L.*) during the winter season. Science 199:796–798. doi:10.1126/science.199.4330.796

Kleiman DG (1966) Scent marking in the Canidae. Symp Zool Soc Lond 18:167–177

Kleiman DG (1974) Scent marking in the binturong, *Arctictis binturong*. J Mammal 55:224–227. doi:10.2307/1379278

Koren L, Mokady O, Geffen E (2006) Elevated testosterone levels and social ranks in female rock hyrax. Horm Behav 49:470–477. doi:10.1016/j.yhbeh.2005.10.004

Kwak J, Willse A, Matsumura K, Opiekun MC, Weiguang Y, Preti G, Yamazaki K, Beaucahmp GK (2008) Genetically-based olfactory signatures persist despite dietary variation. PLoS ONE 3, e3591. doi:10.1371/journal.pone.0003591

Kwak J, Grigsby CC, Preti G, Rizki MM, Yamazaki K, Beauchamp GK (2013) Changes in volatile compounds of mouse urine as it ages: their interactions with water and urinary proteins. Physiol Behav 120:211–219. doi:10.1016/j.physbeh.2013.08.011

Liu D, Yuan H, Wang Z, Wei R, Zhang G, Sun L (2013) Do urinary chemosignals code for sex, age, and season in the giant panda, *Ailuropoda melanoleuca*? Chem Signals in Vertebrates 12, Springer: 207–222. doi: 10.1007/978-1-4614-5927-9_16

Macdonald D (1979) Some observations and field experiments on the urine marking behaviour of the red fox, *Vulpes vulpes L*. Z Tierpsychol 51:1–22. doi:10.1111/j.1439-0310.1979.tb00667.x

MacDonald DW (1980) Patterns of scent marking with urine and faeces amongst carnivore communities. Symp Zool Soc Lond 45:107–139

McCune B, Grace JB, Urban DL (2002) Analysis of Ecological Communities, MjM software design Gleneden Beach, OR

Mertl-Millhollen AS, Goodmann PA, Klinghammer E (1986) Wolf scent marking with raised-leg urination. Zool Biol 5:7–20. doi:10.1002/zoo.1430050103

Moresco A, Larsen RS (2003) Medetomidine-ketamine-butorphanol anesthetic combinations in binturongs (*Arctictis binturong*). J Zool Wildl Med 34:346–351. doi:10.1638/03-016

Müller-Schwarze D (2006) Chemical ecology of vertebrates. Cambridge University Press, Cambridge

Peters RP, Mech LD (1975) Scent-marking in wolves: Radio-tracking of wolf packs has provided definite evidence that olfactory sign is used for territory maintenance and may serve for other forms of communication within the pack as well. Am Sci: 628–637

Petty, JMA (2015) Understanding female social dominance: comparative behavioral endocrinology in the genus *Eulemur*. Doctoral dissertation, Duke University

Petty JMA, Drea CM (2015) Female rule in lemurs is ancestral and hormonally mediated. Sci Rep 5:9631. doi:10.1038/srep09631

Pocock RI (1915) On the feet and glands and other external characters of the viverrinae, with the description of a new genius. Proc Zool Soc Lond 85:131–149. doi:10.1111/j.1469-7998.1915.00131.x

Pocock RI (1939) The Fauna of British India, including Ceylon and Burma. Mammalia, 2nd edn, I. Taylor & Francis, London, U.K

Porton I (1983) Bush dog urine-marking: its role in pair formation and maintenance. Anim Behav 31:1061–1069. doi:10.1016/S0003-3472(83)80013-X

Raeside J (1979) Seasonal changes in the concentration of estrogens and testosterone in the plasma of the stallion. Anim Reprod Sci 1:205–212. doi:10.1016/0378-4320(79)90002-2

Ralls K (1971) Mammalian scent marking. Science 171:443–449. doi:10.1126/science.171.3970.443

Raymer J, Wiesler D, Novotny M, Asa C, Seal US, Mech LD (1984) Volatile constituents of wolf (*Canis lupus*) urine as related to gender and season. Experientia 40:707–709. doi:10.1007/BF01949734

Romanczyk LJ, McClelland CA, Post LA, Aitken WM (1995) Formation of 2-acetyl-1-pyrroline by several *Bacillus cereus* strains isolated from cocoa fermentation boxes. J Agric Food Chem 43:469–475. doi:10.1021/jf00050a040

Rothman RJ, Mech LD (1979) Scent-marking in lone wolves and newly formed pairs. Anim Behav 27:750–760. doi:10.1016/0003-3472(79)90010-1

Schieberle P (1991) Primary odorants in popcorn. J Agric Food Chem 39:1141–1144. doi:10.1021/jf00006a030

Scordato ES, Dubay GR, Drea CM (2007) Chemical composition of scent marks in the ringtailed lemur (*Lemur catta*): glandular differences, seasonal variation, and individual signatures. Chem Senses 32:493–504. doi:10.1093/chemse/bjm018

Sillero-Zubiri C, Macdonald DW (1998) Scent-marking and territorial behaviour of Ethiopian wolves *Canis simensis*. J Zool 245:351–361. doi:10.1111/j.1469-7998.1998.tb00110.x

Staples EJ, Viswanathan S (2005) Odor detection and analysis using GC/SAW zNose®. Electronic Sensor Technology, School of Engineering and Technology, National University

Story HE (1945) The external genitalia and perfume gland in *Arctictis binturong*. J Mammal 26:64–66

Swiecicka I (2008) Natural occurrence of *Bacillus thuringiensis* and *Bacillus cereus* in eukaryotic organisms: a case for symbiosis. Biocontrol Sci Technol 18:221–239. doi:10.1080/09583150801942334

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, Jégou B, Jensen TK, Jouannet P, Keiding N (1996) Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(Suppl 4):741

Weldon PJ, Gorra MF, Wood WF (2000) Short-chain carboxylic acids from the anal glands of the binturong, *Arctictis binturong* (Viverridae, Mammalia). Biochem Syst Ecol 28:903–904. doi:10.1016/S0305-1978(99)00128-3

Wemmer C, Murtaugh J (1981) Copulatory behavior and reproduction in the binturong, *Arctictis binturong*. J Mammal: 342–352. doi: 10.2307/1380710

Wong KC, Lim CL, Wong LL (1992) Volatile flavour constituents of Chempadek (*Artocarpus polyphema* Pers.) fruit and jackfruit (*Artocarpus heterphyllus* Lam.) from Malaysia. Flavour Fragr J 7:307–311. doi:10.1002/ffj.2730070604

Wood WF, Copeland JP, Yates RE, Horsey IK, McGreevy LR (2009) Potential semiochemicals in urine from free ranging wolverines (*Gulo gulo* Pallas, 1780). Biochem Syst Ecol 37:574–578. doi:10.1016/j.bse.2009.09.007

Wynne-Edwards KE (2001) Hormonal changes in mammalian fathers. Horm Behav 40:139–145. doi:10.1006/hbeh.2001.1699

Yoshihashi T (2002) Quantitative analysis on 2-acetyl-1-pyrroline of an aromatic rice by stable isotope dilution method and model studies on its formation during cooking. J Food Sci 67:619–622. doi:10.1111/j.1365-2621.2002.tb10648.x

Zhang J, Soini H, Bruce K, Wiesler D, Woodley S, Baum M, Novotny M (2005) Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. Chem Senses 30:727–737. doi:10.1093/chemse/bji065