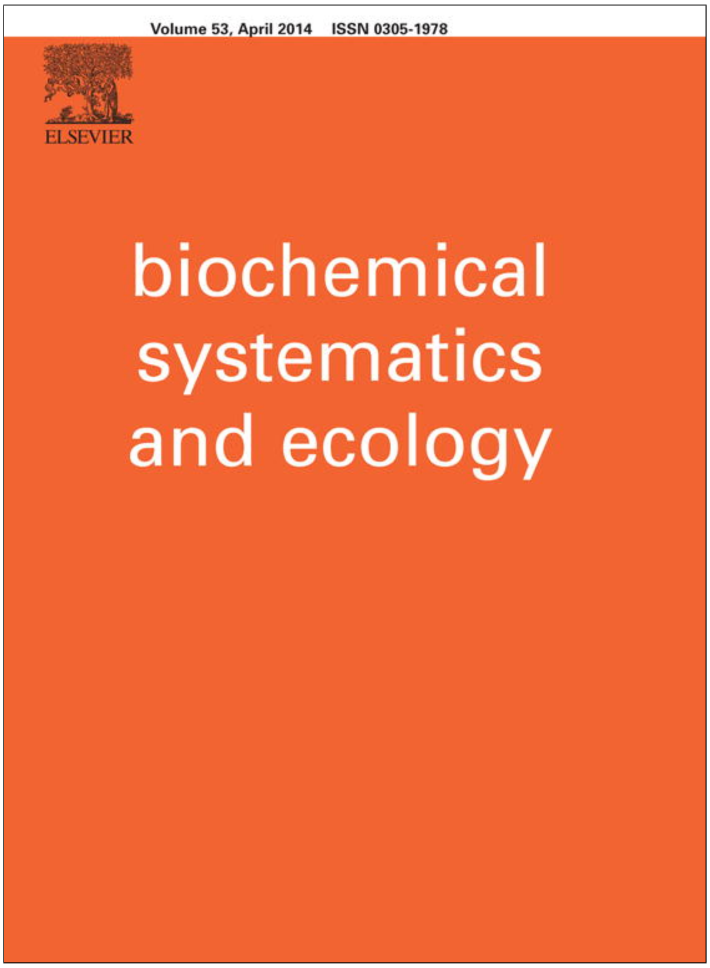
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The “tomcat compound” 3-mercapto-3-methylbutanol occurs in the urine of free-ranging leopards but not in African lions or cheetahs

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A B S T R A C T

The felid-specific urinary odour compound 3-mercapto-3-methylbutanol and its precursors have been found in several felid species in captivity, but its presence in wild felids has not previously been investigated. We analysed the naturally deposited scent marks from three species of wild, free-ranging big cats in Northern Botswana and found 3-mercapto-3-methylbutanol in four samples of leopard urine ($N = 13$), but not in lion urine ($N = 15$) or cheetah urine ($N = 6$). Individual variation in the presence of the tomcat compound in samples from big cats in the wild may reconcile conflicting results from captive cats.

1. Introduction

3-Mercapto-3-methylbutanol is an intensely odorous component of tomcat (*Felis silvestris catus*) urine. It is produced by hydrolysis of the felid-specific amino acid felinine, whose production is catalysed by cauxin, a carboxylesterase enzyme that is unusually abundant in cat urine (Miyazaki et al., 2006b). Urinary felinine levels are highest in intact adult males, they decline after castration and are restored by testosterone injections (Hendriks et al., 1995a,b; Tarttelin et al., 1998; Miyazaki et al., 2006a; Hendriks et al., 2008). Felinine and/or 3-mercapto-3-methylbutanol are widely supposed to be involved in territorial scent marking, but direct biological evidence for this role is lacking.

Cauxin has been reported from a tiger (*Panthera tigris*), an Asiatic lion (*Panthera leo persica*), a clouded leopard (*Neofelis nebulosa*), a Persian leopard (*Panthera pardus saxicolor*) and a jaguar (*Panthera onca*) (McLean et al., 2007), and from bobcats (*Lynx rufus*) and Eurasian lynx (*Lynx lynx*) (Miyazaki et al., 2006a) as well as domestic cats. Felinine has been reported from the domestic cat, ocelot (*Leopardus pardalis*), leopard, Indian leopard cat (*Prionailurus bengalensis*) and bobcat (Hendriks et al.,

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1995a), and 3-mercapto-3-methylbutanol from domestic cats (Miyazaki et al., 2006b) and in a composite urine from bobcats (*Lynx rufus*) (Mattina et al., 1991). Cauxin, felinine and 3-mercapto-3-methylbutanol have not been found outside the felidae, and within the family cauxin is more abundant in small cats than in *Panthera* (McLean et al., 2007; Li et al., 2011) but for some species the results from different studies are inconsistent. All analyses have been of samples from captive animals; no results are available for the occurrence of 3-mercapto-3-methylbutanol in wild, free-ranging felids. To eliminate possible artifacts of captivity we analysed samples from wild African lions (*P. leo leo*), leopards (*P. pardus pardus*) and cheetahs (*Acinonyx jubatus jubatus*). We analysed larger numbers of samples than have been investigated hitherto in order to reduce the effects of individual variability on the reported presence or absence of cauxin, felinine and 3-mercapto-3-methylbutanol.

2. Study area and methods

We collected and analysed urine scent marks from African lions, leopards and cheetahs as part of a larger study (Apps et al., 2012, 2013). Samples were collected in northern Botswana from the Moremi Game Reserve and adjacent wildlife management areas east of the Reserve. This is one of few areas that still support an intact large predator guild and its prey at close to historical densities. As an additional confirmation of compound identity and method validity, one sample from a free roaming, intact, domestic tomcat (*Felis silvestris catus*) was collected in the nearby village of Maun.

Urine spray marks were collected soon after individuals were seen to mark and leave the immediate vicinity. Urine-moistened soil and vegetation were collected using metal implements into cleaned glass jars with lids lined with aluminium foil. In addition, some cheetah scent marks were collected from marking trees (Eaton, 1974; Marnewick et al., 2006) by scraping the surface layer into 22 ml glass vials with foil-lined caps. The tomcat sample was swabbed from a painted wall with filter paper and stored in a 22 ml vial with foil-lined cap. All samples were frozen on the day of collection and kept frozen until thawed for analysis.

In the laboratory, samples were thawed and air dried at room temperature (20–25 °C). Dried subsamples were extracted with the minimum quantity of methanol (Sigma–Aldrich Chromasolv) by steeping them in methanol for 20–30 min and then filtering the methanol through a small pledget of glass wool. Usually 0.2–0.5 ml of extract was collected. Extracts were analysed on a Varian (now Bruker) 450 GC with a 30 m × 0.32 mm × 0.5 μm polyethylene glycol (PEG) capillary column (Restek Rtx-Wax #12349) coupled to a Varian (now Bruker) 320 MS single quadrupole mass spectrometer. 70 eV electron impact mass spectra were tentatively identified by searches of the NIST 05 MS library. The presence of sulphur in compounds was confirmed by running the same extracts on a 30 m × 0.25 mm × 0.5 μm PEG column in a Bruker 450 GC, with detection by flame ionisation and pulsed flame photometric detector in the sulphur mode. Identities were confirmed by co-injection of authentic compounds (Sigma–Aldrich). More details on methods are given in Apps et al. (2012).

3. Results

The "tomcat compound" 3-mercapto-3-methylbutanol was identified in some samples of urine from leopards of both sexes but not in samples from cheetahs or lions (Table 1).

4. Discussion

Our results from wild African lions are consistent with the absence of 3-mercapto-3-methylbutanol and cauxin from the urine of captive lions of unspecified subspecies (Andersen and Vulpius, 1999; Miyazaki et al., 2006a). Nonetheless, McLean et al. (2007) found cauxin in the urine of a single captive Asiatic lion, and it is possible that the 3-mercapto-3-methylbutanol pathway differs between Asiatic lions (*P. leo persica*) and African lions (*P. leo leo*). Our results for wild cheetah are consistent with those of Burger et al. (2006), who did not find 3-mercapto-3-methylbutanol in urine from a captive cheetah. For these two species at least there is no evidence of an effect of captivity.

If the pathway to 3-mercapto-3-methylbutanol in leopards is the same as in domestic cats, then its presence in some wild leopards implies that they also produce cauxin and felinine, and conversely that the quantities of cauxin in some wild leopards are high enough for the production of detectable quantities of 3-mercapto-3-methylbutanol. This is inconsistent with statements by Li et al. (2011), that Pantherids do not produce felinine which is the direct precursor of 3-mercapto-3-

Table 1
The number of samples from each sex of each species of African big cat that contained the "tomcat compound" 3-mercapto-3-methylbutanol.

Species	Sex	Number of samples/number of donors	Occurrence of 3-mercapto-3-methylbutanol
Lion	Male	10/8	Not found
	Female	5/5	Not found
Leopard	Male	11/7	Present in 2 of 5 samples from one male, 1 of 1 sample from another male, absent from 5 samples from 5 males
	Female	2/1	Present in 1 of 2 samples from 1 female
Cheetah	Male	6/4	Not found
Domestic cat	Male	1/1	Present in 1 sample from 1 male

methylbutanol. Results from captive leopards for components of the cauxin – felinine – 3-mercapto-3-methylbutanol pathway are inconsistent: McLean et al. (2007) detected cauxin, and Batta and Harris (1953) detected felinine, but Poddar-Sarkar and Brahmachary (2004) found no 3-mercapto-3-methylbutanol, Miyazaki et al. (2006a) found no cauxin, and Roberts (1963) found no felinine. Our finding that 3-mercapto-3-methylbutanol occurs inconsistently, even within individual leopards, may partially explain the differences among these studies. Similar discrepancies between McLean et al. (2007) and Miyazaki et al. (2006a) in the occurrence of cauxin in lions, tigers and jaguars are likely to be due to differences in the sensitivity and selectivity of their methods, but could also be due to limited sample sizes; results from single samples or from only one or a few individuals might be expected to miss a compound whose presence varies between and within individuals. To clarify whether the occurrence of cauxin, felinine and 3-mercapto-3-methylbutanol correlates with felid systematics will require the analysis of samples from additional species in numbers that are large enough to accommodate inter- and intra-individual variability.

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