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Urinary Volatile Constituents of the Lion, *Panthera leo*

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Abstract

The volatile components of urine from lions were investigated using GC-MS headspace techniques. Fifty-five compounds were found in the urine samples. Seven potential species-identifying compounds were found. Male lion scent marks overlapped significantly more in compound composition with other males than they did with female marks. A similar relationship was not found for the females. Males had a significantly higher absolute content of 2-butanone in their urine than females, and females had a significantly higher relative content of acetone than males. Samples from 13/16 individual lions overlapped more within the individual than they did with samples from the other individuals, but only seven significantly so.

Introduction

The use of chemical compounds in the transfer of signals and messages is an almost universal phenomenon in all living organisms. On a cellular level this process is often very straightforward, but for the inter-individual communication between animals the process is much more complicated. When scent marking occurs in the larger mammals a whole cocktail of chemical compounds is released at the same time, and the relative concentrations of individual constituents may be significant for the meaning of the message transferred.

The secretions of the various glands and the different body metabolites contain a wide range of chemical compounds such as amines, aldehydes, ketones, carbohydrates, alcohols, phenols, fatty acids and esters. The optimal method for detecting each of these classes will depend on their specific chemical properties, and no one method will be optimal for them all. The biologically active compounds may belong to any of these classes. Several studies have produced results on the chemical composition of secretions from various species, but very few of them have been able to pinpoint and classify the biologically active compounds.

The scent communication of mammals has been studied by numerous workers over the last 50 years, but it is only within the last 20 or so years that chemical analyses have become incorporated into these studies on a routine basis. The literature on scent communication in mammals has been reviewed by Bossert and Wilson (1963), Eisenberg and Kleiman (1972), Johnson (1973), Thiessen and Rice (1976), Wemmer and Scow (1977), Brown (1979), Macdonald (1980), Halpin (1980, 1986), Gosling (1982), Albone (984), Jannett (1984) and Brown and Macdonald (1985).

In carnivorous species few detailed studies have been conducted. Two species which have been well studied are the wolf (*Canis lupus*) and the red fox (*Vulpes vulpes*). Raised leg urination (RLU) is considered the most important form of scent communication in the wolf, making up 60–80% of all scent marks observed (Peters and Mech, 1975). Raymer *et al.* (1984, 1986) identified 77 different compounds in the bladder urine of wolves. Castration of male wolves led to a decline in the concentration of most compounds as well as in the actual number of compounds. Asa *et al.* (1990) found that serum testosterone levels were positively correlated with urine marking rates in dominant wolves of both sexes.

In the red fox the most common form of scent marking has been termed token urination. The token urinations, in which the animal sprinkles a few drops of urine, are distinguished from normal squat urinations by their brevity and the choice of urination site. The chemical composition of fox urine was first studied by Jorgenson *et al.* (1978). The urine was collected from snow on which the foxes had marked. They identified a range of different compounds, including four which appeared to be species-specific for the fox. Another compound (2-methylquinoline) was found in male urine only. Wilson *et al.* (1980) tested a solution made from synthetic copies of eight of the compounds identified by Jorgenson *et al.* (1978) against a control solution. They found that the test solution provoked significantly more counter-marking by wild foxes than did the control solution (also reported in Whitten *et al.*, 1980). Bailey *et al.* (1980)

studied the volatile part of fox urine and found a possible seasonal fluctuation for some of the compounds identified. In contrast to Jorgenson *et al.* (1978), they also found 2-methylquinoline in the urine of female foxes.

Controversy has long reigned over the precise origin of the 'marking fluid' used by lions (*Panthera leo*) and tigers (*P. tigris*) in scent marking. It has been reported by several workers that when these big cats deposit scent marks they include secretions from the anal sacs in the scent mark (lion: Schaller, 1972; Bertram, 1978; tiger: Schaller, 1967; McDougal, 1977; Brahmachary and Dutta, 1981). This conception was formed on the basis of the observation of 'a granular, whitish precipitate . . . apparently a secretion from the anal glands' being present in the marking fluid of the tiger by Schaller (1967). This misconception was finally rectified by Brahmachary and Dutta (1987) when they concluded that as there is no connection between the anal glands and the urinary tract (Hashimoto *et al.*, 1963), the anal gland secretion can only mark the faeces and not the urine. The 'whitish precipitate' must therefore originate somewhere from inside the urinary system. Hewer *et al.* (1948) investigated the contents of the bladder of a dead tiger and found substantial amounts of lipids in the bladder urine. They also found lipids in the urine collected from the floor of night cages housing tigers of both sexes. They estimated that tigers excrete as much as 20 g of lipid matter a day, and that the concentration of lipids in the urine is correlated with the kidney fat index rather than with the level of lipids in the blood. Asa (1993) carried out a series of analyses on bladder urine extracted from anaesthetized animals which confirmed that the bladder urine of lions and tigers contain lipids in the form of a whitish substance. She did not find evidence of lipids in the bladder urine of the leopard (*P. pardus*), puma (*Felis concolor*) or cheetah (*Acinonyx jubatus*).

All cat species have two distinct behavioural patterns to deposit fluid from the bladder via the urinary tract. These two patterns are normal *urination*, in which the animal squats on the ground and releases a stream of urine, and *spraymarking*, in which one or more sprays of urine is ejected by the animal while it stands with its hindquarters towards the marked object with tail lifted vertically. The fluid deposited in both these behaviours comprises normal urine mixed with a whitish lipid precipitate also present in the bladder. When deposited as normal urination the lipids do not leave distinct marks on the ground but when sprayed on vertical objects the lipids will stick to the surface and in time turn into a black tar-like substance which marks the object for a long time. This effect is intensified as the animals tend to spraymark the same objects at regular intervals. No quantitative analysis of the lipid content of tiger and lion urine has been presented in the literature, but from the authors' own observations it appear that tiger urine has a much higher content of lipids than lion urine. Indeed, even though lion urine contains lipids (Asa, 1993), their spraymarks does not leave the same black long-lived deposits of lipids as tiger marks do.

As lipids are present in the bladder urine they will be released both by spraymarking as well as by normal urination. It is therefore unlikely that their presence in scent marks is the result of a voluntary process involved in the motor pattern of scent marking. It is also doubtful whether an animal is able to control the ratio of 'lipids to urine' deposited in a scent mark. Therefore no circumstantial or conclusive evidence has been presented so far for the addition of any secretions to the bladder urine before it is deposited as a scent mark. Against this background it is reasonable to assume that the bladder urine from an animal and therefore its urine produced during a normal urination will contain exactly the same range of chemical compounds as that found in the spray mark once it is deposited. No studies have yet described the composition of the lion urine used in scent communication. This task was undertaken in the study presented here. Furthermore, an effort was made to find qualitative and quantitative differences between the scent marks of individuals and sex groups.

Materials and methods

The study animals from which urine samples were collected were located in two different Danish zoos or safari parks. One was København Zoo (KBH), which is an urban zoo located in the capital of Denmark, København. The second was Givskud Safari Park (GIV), which is situated in Jylland. In addition, Aalborg Zoo in Jylland and Kolmaarten Safari Park in Sweden provided urine samples from one male lion each.

Collection of scent samples had to be adjusted to the individual conditions in the enclosures and cages. For the KBH lion group and the Aalborg group samples were collected from the floor of the night cages. In GIV samples were also collected from the floor of the night cages, but as these were mostly covered in sawdust, a method had to be devised for extracting the samples from the sawdust. This was done by constructing an oversized 'garlic press' of stainless steel which could hold about one litre of sawdust, and by compressing this, the fluid could be extracted and collected in test tubes.

Altogether, 19 samples from male lions were analysed. These originated from five adult males (18 samples) and one sample from a male lion cub. A total of 44 female lion samples were analysed. These were from 13 adult female lions and one sample of bladder urine from a sub-adult female. All samples originated from healthy normal individuals and to the authors' knowledge none of the females were in oestrus or pregnant at the time of sampling.

All samples were stored in identical plastic test tubes with a holding capacity of 10 ml. The samples were frozen immediately after collection and kept at –18°C until the time of analysis.

Chemical analysis

The headspace technique provides a chemical profile of the volatile compounds which evaporate into, and form an equilibrium with, the air above the actual liquid sample, the so-called headspace, and thus provides information on the compounds which would be picked up by the nose of a passing animal.

All chemical analyses were performed on a combined gas chromatograph–mass spectrometer (Fisons MD 800). Undiluted urine (1 ml) was introduced into a headspace vial with appropriate labelling, which was then placed in the holding tray of the headspace autosampler of the chromatograph. The carrier gas used was helium at an inlet pressure of 5 psi.

The technical specifications of the GC capillary column was: column ID: J & W, DB624 (4320732); film thickness: 1.80 μm; column dimensions: 30 m × 0.315 mm; temperature limits : –20 to 260°C; coating: 6% cyanopropylphenylsilicone and 94% dimethylsilicone.

The analyses were run automatically using the MassLab (v1.1) computer program. The chromatograph was run in a split mode with a split ratio of 1:5 and the following additional parameters: injector temperature 200°C; initial oven temperature 40°C for 1 min; this was then increased by 10°C/min up to 200°C and this temperature was maintained for 8 min. The headspace vial was preheated for 1 h at 70°C before the headspace sample was taken and injected into the chromatograph. The amount of sample air injected in each case was 1 ml.

The spectrometer was run with the following parameters: type: full scan; ionization mode EI+, Mass range: *m*/*z* 20–350.

When transferring the scent sample into the headspace vial it is inevitable that molecules present in the laboratory air at that particular time also become included in the sample. These laboratory air compounds will then contaminate the test sample by appearing as peaks on the chromatogram resulting from the analysis. In order to minimize the impact of this contamination on the final results, a set of two laboratory air samples were also analysed each time a test series was analysed. The peaks caused by the compounds in the laboratory air can thus be subtracted from the chromatograms of the test samples. Another possible source of contamination for most of the lion samples are compounds originating from the sawdust from which the urine samples were extracted. To eliminate the influence of this contamination on the results, three extractions of distilled water on sawdust were made, one being allowed to soak in the sawdust for 1 h, the second for 3 h and the last for 5 h. These extractions were then analysed using the same procedure as for the urine samples, the resulting chromatograms making it possible to identify compounds with a probable origin from the sawdust and thus to exclude those compounds from the further data analysis.

The GC-MS data for each of the analysed samples were stored on disk and later analysed with the MassLab programme. For each sample a total ion chromatogram was produced; for each of the peaks in the chromatograms the corresponding mass spectrum was extracted and printed. From the mass spectra the corresponding compounds were identified. The identification was made using standard interpretation principles (McLafferty and Tureček, 1993) and with the help of the NIST/EPA/NIH Mass Spectral Database v4.0.

The total ion count (TIC) was extracted for each of the peaks on the chromatograms by measuring the area underneath, and these values were used to approximate the relative content of each compound in a sample. Both the absolute and relative peak areas (TIC) were used in a quantitative analysis of differences between the sexes.

In comparing the scent samples, the concept of 'overlap' was used in some of the analyses. An overlap is defined as the percentage of the total number of compounds present in an individual sample which are common to both individuals (or samples) being compared; for example, if two lion males have 10 compounds in common between their respective samples, and the first male has a total of 20 compounds present in his samples and the second male 15, then the overlap from the first male's point of view will be 50% whereas the second male will experience a 67% overlap.

Results

Compounds identified in lion urine

On the basis of the identification process of the mass spectra a list of 81 different compounds was produced, each of them being found in one or more of the samples analysed. Of these 81 compounds 18 were also identified in the accompanying control samples of laboratory air, and a further eight were also found in the control sawdust extractions. Thus 55 compounds can with certainty be said to originate from the lion urine whereas the other 26 might have had different origins. All 81 compounds are listed in Tables 1 and 2 with an indication of their suspected origin. A number of the identifications are tentative and require further work for confirmation. Table 1 also lists the number of samples analysed for each individual and the total number of compounds identified for these individuals excluding those compounds which were also found in corresponding control samples of laboratory air and sawdust extraction. These latter compounds have been excluded from the following analysis. As can be seen from Table 1, the samples from the two lion males Napoleon and KolmHan contained far fewer compounds than the rest of the lion samples. The samples from these two males were not collected by the authors, but sent to us by the keepers of the respective zoos. Instructions were given by us to the

Table 1 Compounds present in the urine of male ($n = 5$) and female ($n = 13$) lions

No.	Retention time	Name	Males					Females												
			Pol	Ner	Flo	Nap	KolM	Lin	Fif	Len	Fie	Gur	Nan	Fio	Nat	Npo	Nel	Net	Lil	Nin
1	1.126	trimethylamine			X	X	X			X			X		X	X	X	X	X	X
2	1.376	pentane	O	O	O	O		O	X	X	O	O	O	O	O	O				
3	1.426	ethanol							X	X										
4	1.642	acetone	O	O	X	O	X	O	O	O	O	O	O	O	X	O	X	X	X	X
5	1.742	carbon disuphfide	O	O	O	O		O	O	O	O	O	O	O	O	O				
6	1.909	methylene chloride	O	O	O	O		O	O	O	O	O	O	O	O	O				
7	2.093	dimethyl ether		O					O				O							
8	2.292	hexane	X	X	X				O	O			O		X					
9	2.442	diisopropyl ether	O	O	O	O		O	O	O		O	O		O	O				
10	2.809	2-butanone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
11	3.026	tetrahydrofuran (THF) + chloroform		O					O				O							
12	3.493	benzene + column bleeding		O					O	O			O							
13	3.593	2,2,3,3-tetramethylbutane		O									O							
14	3.610	3-methylbutanal	S		S		X	S							S				S	S
15	3.726	2-methylbutanol								X										
16	3.776	heptane											X							
17	3.793	?	X																	
18	4.093	trichloroethylene		O					O	O			O							
19	4.260	S-methyl thioethanoate + coelute							X	X										
20	4.293	2-pentanone	X	O	X			X	X	X					X	X	X	X	X	X
21	4.360	3-methyl-1-butylamine				X	X													
22	4.393	pentanal	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S
23	4.443	3-pentanone (?)		X																
24	4.576	bromodichloromethane		O	S				O	O			O							
25	4.610	1-pentene	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
26	5.076	dimethyl-disulphide					X								X		X	X		X
27	5.410	toluene	O	X	X			O	O	O	O	O	X	O	X	O	X	X	X	X
28	5.893	1-methoxyethanethiol	X	X	X			X	X	X	X	X	X	X	X	X				
29	6.043	3-hexanone	X	X	X							X								
30	6.043	tetrachloroethylene		O						O			O							
31	6.110	cyclohexanone	O	O	O			O	O	O	O	O	O	O	O	O				
32	6.340	hexanal		X					X	X			X							
33	6.343	dibromochloromethane + coelute											O							
34	6.343	?	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S
35	6.610	2-pentylfuran	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
36	7.094	chlorobenzene		O									O							
37	7.277	ethylbenzene		O									O							
38	7.427	p-xylene		O					O	O			O							
39	7.627	1-octanal			X				X				X							
40	7.727	4-heptanone					X					X	X							
41	7.744	2-butylfuran	X	X	X			X	X	X				X						
42	7.927	3-methyl-1-pentanol	X	X	X			X	X			X								
43	7.960	o-xylene + styrene		O									O							
44	8.177	tribromomethane + methyl-ketone		O									O							
45	8.177	methyl-ketone (?)	S	S	S		X	S	S	S		S	S	S	S	S	S	S	S	S
46	8.210	2-heptanone							X				X							
47	8.275	heptanal	X	X	X			X	X	X	X	X	X	X	X	X		X	X	X
48	8.277	1.2-cyclooctanediene + ?	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
49	9.777	benzaldehyde	O	X	X			O	X	X	O	O	X	O	X	O	X	X	X	X
50	9.861	2.3-octanedione + coelute													S					

Table 1 Continued

No.	Retention time	Name	Males					Females											
			Pol	Ner	Flo	Nap	KolM	Lin	Fif	Len	Fie	Gur	Nan	Fio	Nat	Npo	Nel	Net	Lil	Nin
51	9.911	1.3-octadiene + branched C9 alkane		X				X	X				X	X						
52	10.211	?	X	X	X			X	X	X	X	X		X	X		X	X	X	X
53	10.228	octanal	X	X	X			X	X	X	X	X	X	X	X	X				
54	10.961	3-ethyl-2-methyl-1,3-hexadiene			X			X	X				X	X	X		X		X	X
55	11.211	alkane								X		X			X	X				
56	11.245	2.2-dimethyl-3-hexanone								X										
57	11.511	(E)-2-octenal	S	S	S			S	S			S		S						
58	11.811	1-phenyl-1-pentanone (?) (C11H14O isomer)		X					X		X	X								
59	11.995	nonanal	X	X	X			X	X	X		X	X	X	X	X		X	X	X
60	12.045	methylbenzoate + coelute	X	X	X			X			X	X								
61	12.912	dodecane			X					X		X			X	X	X	X	X	X
62	13.295	diethylbenzene	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
63	13.528	?	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S
64	13.778	O-isopropenyltoluene (?) (C10H12 isomer)	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S
65	14.495	alkane								X		X			X	X	X		X	X
66	16.796	1.2-dimethoxy-4-(2-propenyl)-benzene	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S
67	18.162	1,1′-(1,3-phenylene)bis-ethanone	S						S					S	S					
68	18.179	dimethyl phthalate[a]								X						X				
69	18.396	butylated hydroxytoluene	O																	
70	18.546	1,4-dihydro-1,4-ethenonaphthylene[a]				X														
71	20.947	diethyl phthalate[a]								X		X			X	X				
Number of samples analysed:			4	4	5	2	3	3	4	3	1	3	2	2	7	1	5	4	4	4
Number of compounds (excluding controls and sawdust):			17	21	23	4	8	16	23	25	11	19	19	13	22	16	15	15	16	17

X denotes the presence of the compound in one or more of the samples analysed. O indicates that the compound was present in both the sample and in a corresponding control sample (laboratory air). S indicates that the compound was present in both the sample and in the 'water–sawdust' extraction. At the bottom of the table the number of samples analysed for each individual is shown as is the total number of compounds found (excluding those found in laboratory air controls and 'water–sawdust' extraction samples). 'Retention time' is defined as the time taken for each analyte to emerge from the chromatographic column (here given in minutes).

[a]Contamination from plastic test tubes?

keepers on how to collect and keep the samples, but it is not known to what degree these instructions were followed. Furthermore, the samples were exposed to normal room temperatures during the period in the post, and these two factors may go some way to explaining the obvious difference in compound numbers. The samples from these two individuals are included in Table 1 because new compounds were identified in them. However, due to the difference in the total number of compounds found in these two males relative to the other males, they were excluded from the following inter-individual and sex group comparisons, but included in the intra-individual comparisons as they do not influence the overall results in this case.

Of the compounds identified in the lion urine, numbers 14, 17, 21, 23, 45 and 70 were specific to male lions, although they were not found in all samples from male lions. Compound numbers 2, 3, 15, 16, 19, 46, 55, 56, 65, 68 and 71 were found only in female samples, though again they were not all found in all female samples. The rest were found in one or more samples from both sexes.

From the data in Table 1 an average linkage cluster analysis was performed to clarify any underlying trends in the relatedness of compound composition for the different lions. The resulting dendrogram is shown in Figure 1. Three main clusters are seen but with no obvious separation between males and females.

Table 2 Further compounds found in two additional lion samples—one from a GIV male cub (SG) and one from the bladder of a subadult KBH female (KB2)

No.	Retention time	Name	Found in sample:
72	1.592	azetedine?	KB2
73	2.993	(±)-2-butanol	KB2
74	5.393	3-methyl-1-butanol	KB2
75	5.743	3-methyl-pentane	KB2
76	7.260	4-methyl-3-penten-2-one	KB2
77	9.427	6-methyl-2-heptanone	KB2
78	10.044	2-octanone	SG
79	11.278	phenol	KB2
80	11.828	2-undecanone	SG
81	13.245	?(heptanal?+?)	KB2

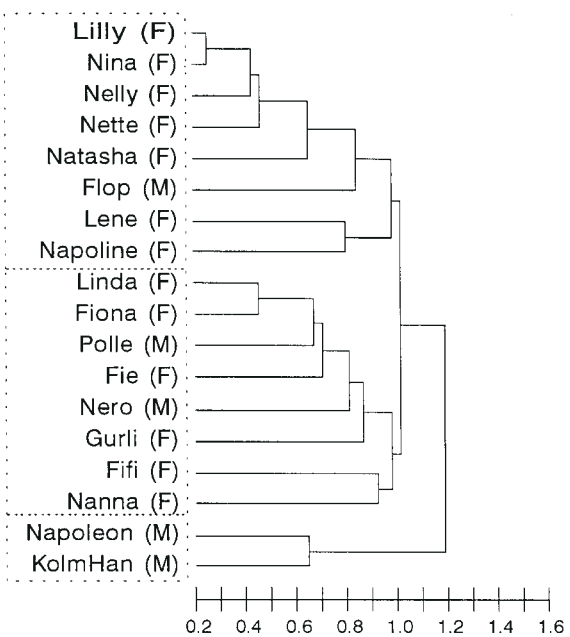

Figure 1 Average linkage cluster dendrogram showing the 'relatedness' between individual lions of the chemical composition of scent marks. The axis at the bottom shows the root-mean-square distance between observations. Note that three main groups are evident (indicated by the dashed boxes), and that the groups are not divided by sex. The two upper groups are all individuals from GIV. The bottom group (Napoleon and KolmHan) comprises individuals from two other zoos. M: male, F: female.

Overlap in compound composition within and between the sex groups

An overlap between two individuals or sex groups is calculated as the number of compounds common to both individuals or groups divided by the total numbers of compounds for each of the individuals or groups (see Material and methods). By comparing all male and female lions in this way total mean values for the male–female and the female–male overlap can be calculated. The same method is used for the calculations within each of the sex groups.

Figure 2 depicts the difference in compound overlap between the sexes (in this calculation the data from Napoleon and KolmHan are omitted). A significant difference was found (t = 4.22, df = 43, $P < 0.001$) between the mean overlap within the male group and the mean overlap between males and females (male–male overlap: n = 6, mean = 79.7, SE = 4.0; male–female overlap: n = 39, mean = 59.9, SE = 1.7). No significant difference was found (t = 1.78, df = 193, P = 0.076) for the corresponding comparison between the mean female overlap and the mean overlap between females and males (female–female overlap: n = 156, mean = 66.5, SE = 1.3; female–male overlap: n = 39, mean = 71.6, SE = 2.6).

Figure 3 shows the mean overlap in compound composition for all different samples from the same individual ('within overlap') compared with the overlap of each individual with the other individuals of the same sex ('between overlap'). A t-test was used to analyse these data for significant differences. From Figure 3 it is evident that two of the males (Polle and Flop) and one of the females (Natasha) had a lower 'within overlap' than 'between overlap'; the rest of the individuals all had a higher 'within overlap' than 'between overlap'. In the male group only KolmHan had a significantly higher 'within overlap' than 'between overlap' (t = 4.82, df = 8, P = 0.001). Six of eleven females had significantly higher 'within overlap' than 'between overlap', namely Linda (t = 3.23, df = 16, P = 0.005), Fifi (t = 7.23, df = 22, $P < 0.001$), Lene (t = 5.81, df = 16, $P < 0.001$), Gurli (t = 5.37, df = 16, $P < 0.001$), Lilly (t = 2.79, df = 22, P = 0.013) and Nina (t = 2.26, df = 22, P = 0.034).

Differences in absolute and relative content of common compounds between the sexes

A number of the compounds listed in Table 1 were found to be present in almost all individuals of both sexes. Excluding the compounds which were also found in the two types of control samples, these compounds were numbers 4, 10, 25, 35, 47, 48 and 62. The absolute content (TIC) of each of these compounds was measured as the areas underneath each of the peaks on the respective chromatograms. The absolute contents of each of the seven compounds for the two sexes are shown in Figure 4. A significant difference in content is seen between the sexes for compound 10 (2-butanone), with males having a much higher content than females (unequal variance, t = 2.50, df = 18.1, P = 0.022). None of the other compounds was found to have a significant difference in content between the sexes.

The relative contents (in %) of each of the seven common compounds were also calculated for male and female lions. The results are shown in Figure 5. Of these common compounds, females were found to have a significantly higher relative content of compound 4 (acetone) than males (t =

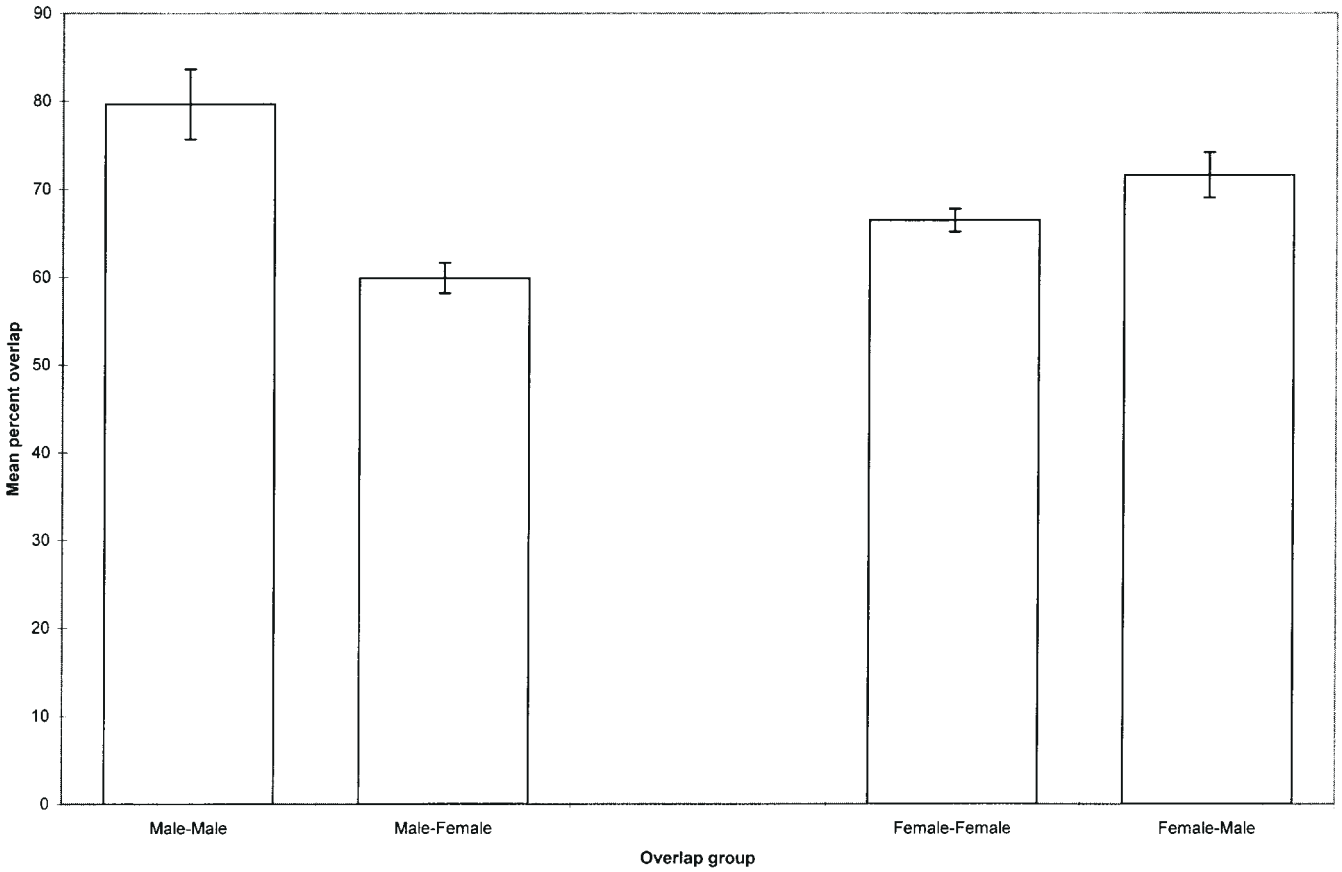

Figure 2 Difference in compound overlap within and between the lion sex groups.

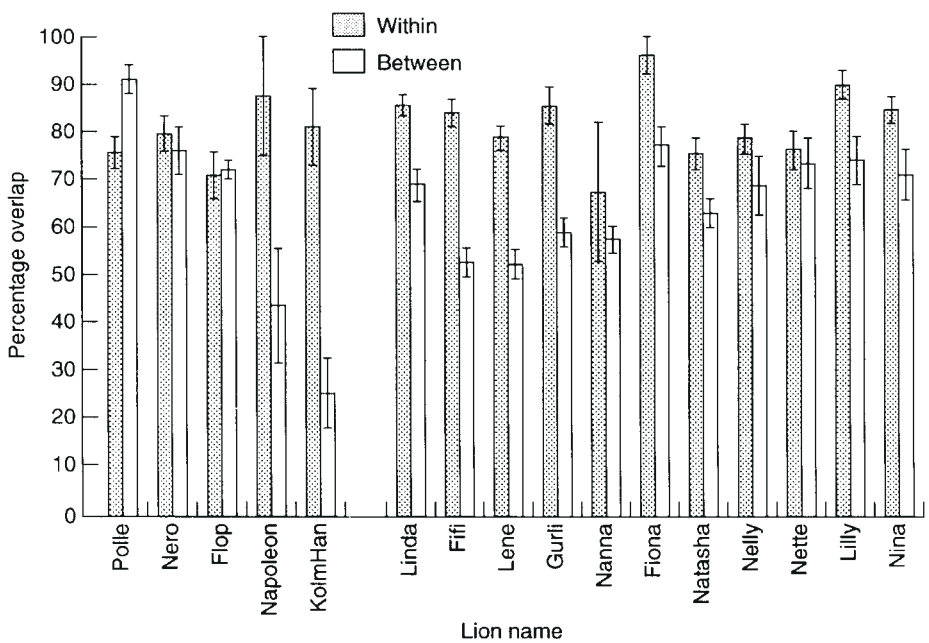

Figure 3 Percentage overlap in compound composition within and between individuals of the same sex groups for lions. Males are shown to the left and females to the right.

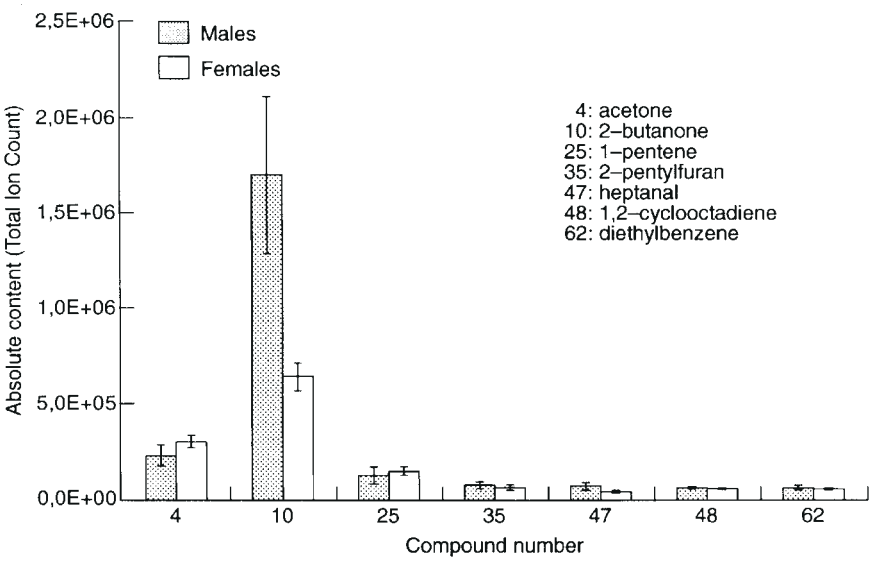

Figure 4 Sex difference in absolute content (area under the peaks of the chromatogram) for different compounds in lion urine.

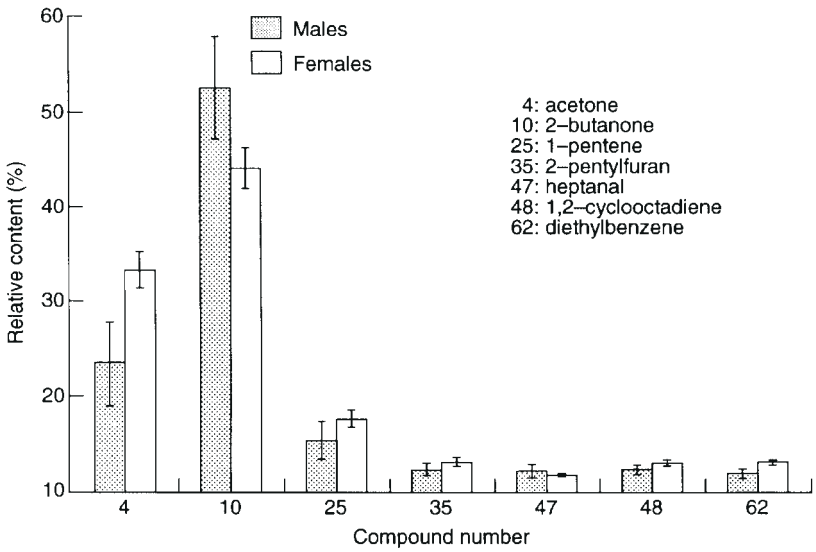

Figure 5 Sex difference in relative content (%) of different compounds in lion urine.

2.32, df = 27, $P = 0.028$). They also had a higher relative content of compounds 25 (1-pentene) and 62 (diethylbenzene) than males, but not significantly so. Males were found to have a higher relative content of compound 10 (2-butanone) than females, though again not significantly so. For the rest of the common compounds there were no major differences in the relative content between the sexes.

Discussion

The group-living lion has three potentially different situations in which chemical signals can be used. Firstly, a scent mark could be deposited in the presence of other members of the pride and intended for communication with those other members, i.e. as an instantaneous communication in which the signal life-time needs only be short. Secondly, as lion groups often split up into smaller units which move independently throughout the territory, a scent mark deposited by a lion in one of these units could be intended for members moving around in the other units, thereby helping them to keep track of each other. This signal would have to last for at least a few hours up to a couple of days in order to be effective. Thirdly, lions may leave scent marks within and on the borders of their territories in order to inform potential intruders that the area is already occupied. These marks would have to last for a long time, weeks if not months, in order to be an effective

means of communication. Supposing the lion is capable of producing only one type of scent mark, the mark would have to be composed of a range of chemical compounds, some with very high, some with medium and some with low volatility.

No published data on chemical composition of lion urine are available. Brahmachary and Dutta (1981) erroneously quote Albone *et al.* (1977) in stating that putrescine (1,4-butanediamine) and cadaverine (1,5-pentanediamine) are present in lion marking fluid, when in fact they wrote (Albone *et al.*, 1977, p. 37): 'In addition, ammonia, putrescine and cadaverine are major contributors to the *anal sac secretions* of both the lion and the red fox' (our italics). However this misunderstanding may be based on the fact that at that time, marking fluid was thought to contain secretions from the anal sacs.

Special attention has been given to amines by other workers when they have analysed scent samples from the *Panthera* species (Brahmachary and Dutta, 1979, 1981, 1987; Banks *et al.*, 1992). Whether or not this special attention is justified in that amines play an important role in the communication system of these species still remains to be seen.

Trimethylamine (no. 1) was detected in samples from three of the five male lions (Table 1) and in eight of the 13 female lions, as well as in the sample of bladder urine from a KBH female (KB2). 3-Methyl-1-butylamine (no. 21) was found in two male lions (Napoleon and KolmHan). Compound 72 (azetidine?) was found once in a sample of bladder urine (KB2, Table 2). From this evidence it appears that at least one amine (trimethylamine) is regularly though not universally present in lion urine. Trimethylamine is a highly volatile compound and it could therefore be used for short-term communication purposes, but whether or not it plays a role in the scent communication system of lions is impossible to say at this stage.

No studies are available on the non-amine fraction of lion urine. The most common of the non-amines found in lion urine in this study were acetone (no. 4), 2-butanone (no. 10), 1-pentene (no. 25), 2-pentylfuran (no. 35), heptanal (no. 47), 1,2-cyclooctadiene (no. 48) and diethylbenzene (no. 62). All of these compounds were detected in most samples from most of the individuals. A whole range of other compounds were present but found much less frequently than those mentioned above. Some of these compounds will be excretions from the normal metabolic process, whereas others will have a different origin. The role, if any, of these individual compounds are still unknown, but a few guesses can be ventured on the basis of the results presented in the previous section.

Can lion scent marks be used in identification on either a species, sex or individual level?

Out of the 55 (81) chemical compounds identified in this study from the lion samples, seven were almost universally present and it is therefore possible that some or all of these compounds could carry a message of species identity individually or in combination. The seven compounds are listed in the paragraph above. Great care has to be taken, however, when such conclusions are drawn. An example of this was shown by Brahmachary and Dutta (1979), who, after having identified phenylethylamine (PEA) repeatedly in tiger urine samples, stated that 'PEA is likely to be a biochemical marker at the species level'. The same authors later found PEA to be present in leopard urine as well (Brahmachary and Dutta, 1984), and since these two species occurs sympatrically it is impossible for PEA alone to act as a marker on the species level.

Of the compounds identified in only male lion samples, none was present in all the males, and it is therefore uncertain whether one or more of these compounds carries a message of 'maleness'. Only if other lions could 'remember' this list of compounds and by the presence of any one of them identify a given scent mark as originating from a male lion could such a system work. It is also conceivable that there exists a universal male identifying compound in the urine which has not been discovered by the method of analysis available to the authors in this study, and that this universal compound could be a derivative of the male hormone testosterone. Such a compound could be part of the involatile part of the urine and still provide important semiochemical information via the vomeronasal organ. As was seen for the male lion samples, the compounds found only in female lion samples were far from common to all the female samples analysed, so again they could only carry a message of 'femaleness' if lions are able to 'remember' all these compounds and by the presence of any one of them are able to identify a scent mark as having been deposited by a female lion.

Though no specific 'male' compound was identified, it was found that males overlapped other males significantly more in compound composition than they did females. However, a similar significant difference was not found for the females, so it is still unclear what role, if any, the overall compound composition plays in the identification of sex.

On the quantitative level a difference was detected between the two sexes in that males had a significantly higher absolute content of 2-butanone (no. 10) than females. Females had a significantly higher relative content of acetone (no. 4) than males. Further minor non-significant differences in the relative content between the sexes were present. If lions are able to detect such differences, a high absolute content of 2-butanone coupled with a low relative content of acetone could signal 'maleness' and the opposite relationship could signal 'femaleness'. Alternatively any of these two factors alone could contribute to the sex identity of lion scent marks. Thus in combination qualitative and quantitative aspects of the scent marks are likely to be able to carry a message of sex identity.

Do the scent marks carry a message of identity for the individual lion? Some evidence for this was found in that no two individuals had an identical compound composition. It is therefore possible that the compound composition of a scent mark alone could hold the key to the identity of the animal. This was tentatively supported by the finding that 13 of the 16 individual lions studied had a higher overlap within their own samples than their total composition overlapped those of the other individuals. However, only seven out of the 13 overlaps were significantly in favour of the 'within overlap', so the conclusion is by no means clear cut.

In the present discussion it is important to remember that the method of analysis available to the authors does not provide a complete chemical profile of lion urine. Therefore it is likely that more compounds will be identified in lion urine as further methods of analysis are employed. Whether the biologically active compounds are present in the fraction identified and listed in this study is not known at the present time, but possible candidates have been identified.

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