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Otter Scent Signals Age, Sex, and Reproductive Status

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Abstract

Scent is used across taxa to communicate information about signaler identity. Eurasian otters *Lutra lutra* are mainly solitary and thought to use scent as their primary means of communication. Little is known, however, about what information otters communicate through scent or what social function this performs. Headspace solid-phase microextraction and gas chromatography–mass spectrometry were used to sample and analyze volatile organic compounds from anal scent gland secretion from 158 otters of differing sex, age, and female reproductive status. Univariate and multivariate differences were clear between adult and juvenile otters. Complex sex differences were apparent in adult otters but not in younger individuals, suggesting the use of this scent secretion in mate attraction. The scent of pregnant and lactating females was highly differentiated from male and juvenile scent, but anecdotal reports suggest females avoid communication during these times.

Key words: gas chromatography, Lutrinae, mass spectrometry, scent communication, SPME, volatile organic compounds

Introduction

Mustelids use scent marks for intraspecific communication and have been the subject of chemical research for over 140 years, probably as a result of the aggressively malodorous nature of their scent marks (Burger 2005). Scent communication is common across social systems providing a means for group cohesion for social species (Buesching et al. 2003) and a means of avoiding costly agonistic encounters with other individuals for solitary species (Erlinge et al. 1982). Hutchings and White (2000) propose 2 main functions of mustelid scent communication; communication of reproductive status and availability and/or use of resources. Chemical analysis of scent marks can provide a useful insight into what information may be communicated about the identity of the signaler. Body condition and reproductive status are communicated through scent by badgers (Buesching, Waterhouse, et al. 2002) and communication of individual identity has been found in a number of mustelid species (Buesching, Waterhouse, et al. 2002; Zhang et al. 2003, 2005). Although age and sex differences in mustelid scent secretions may seem ubiquitous (Zhang et al. 2003, 2005) they are not apparent in all species (Zhang, Zhang, et al. 2002) or are only found at certain times of year (Service et al. 2001).

Otters are a member of the mustelid family but very little is known about the chemical nature of the scent marks of any of the 13 species forming subfamily Lutrinae. Here, we focus on the Eurasian otter (*Lutra lutra*) which throughout much of their range are nocturnal and elusive, making research into their ecology and behavior extremely difficult. Otters are thought to be mainly solitary with typical ranges of up to 40 km (Erlinge 1967, 1968; Green et al. 1984; Kruuk 2006) but can travel even further (Durbin 1998). Encounters with conspecifics are therefore likely to be rare, meaning the use of vocal or visual communication is limited and scent is likely to be a key method of communication. Like most mustelids, Eurasian otters have a pair of anal scent glands that produce a secretion which, when deposited with faeces, is known as spraint. It is unlikely that the glandular secretion is deposited without faeces although it may sometimes be deposited with a jelly like substance from the gut (Trowbridge 1983). Spraint and anal jelly are deposited by otters in a way that is typical of scent marking, for example, they are left in prominent locations (Erlinge 1967). Despite the established use of spraint in surveys (Crawford 2010) and various suggestions regarding the communicative function of spraint, including territory marking (Gosling 1982), resource defence (Kruuk 1992), and mate attraction (Kruuk 2006), the chemical characteristics and messages conveyed in these scent marks remain unknown.

Anecdotal accounts of otters sniffing spraint exist (Gorman et al. 1978; Trowbridge 1983; Kruuk 2006) but these authors do not report otters making direct contact with it. It therefore seems likely that at least part of communication is achieved

through volatile organic compounds (VOCs), as opposed to involatile compounds comparable to, for example, the major urinary proteins used by mice (Hurst et al. 2001; Nevison et al. 2003). Differences in VOCs relating to sex and/or age have been reported in other mustelid species' scent marks, for example, badgers, *Meles meles* (Buesching, Waterhouse, et al. 2002), ferrets, *Mustela furo* (Zhang et al. 2005), Siberian weasel, *Mustela sibirica*, and Steppe polecat, *Mustela eversmanni* (Zhang, Sun, et al. 2002).

Early attempts to investigate *L. lutra* scent and the type of information communicated suggest differences between individuals (Gorman et al. 1978; Trowbridge 1983) but very small sample sizes ($n = 2$, $n = 3$, respectively) limit conclusions. More recent attempts show no clear difference between male and female scent (Bradshaw et al. 2001), but the use of solvent extraction may have diluted some components of the scent below detection limits. To avoid this, a different extraction method is employed in the present study. Furthermore, previous studies focused comparisons only on the most abundant of components detected. This approach may have obscured differences between groups, as the compounds that produce the greatest discrimination between groups are not always the most abundant (Willse et al. 2005). Scents used in communication are complex mixtures and therefore ratios of multiple compounds can provide the necessary information where individual components do not (e.g., Pareja et al. 2009).

The elusive nature of the otter makes collecting spraint from known individuals in the wild impractical, and there are too few otters in captivity to investigate the chemical messages in spraint accurately. This research therefore makes use of otters found dead, which provides a unique opportunity to collect samples from individuals for which complementary parameters such as sex, age, reproductive status, size, and indicators of health (e.g., parasite load and body condition index; Chadwick 2007) can also be recorded. As current survey methods (spraint surveys) assess distribution only (Crawford 2010), and DNA analyses from spraints to identify individuals have a low success rate (Dallas et al. 2003), the potential to use chemical analysis of spraints for estimating the sex ratio and age structure of wild otter populations would offer new and affordable ways for noninvasive population monitoring. In this study, we aim to discriminate between groups of otters (by age, sex, and female reproductive status) based on profiles of VOCs from their anal scent gland secretions.

Materials and methods

Sample collection

Otters found dead in England and Wales are collected for postmortem examination by Cardiff University Otter Project. Individuals deemed to be fresh (showing little sign of autolysis) were selected to represent different sexes, age-groups, and (for females) reproductive status (n = 158, Table 1). Anal glands are removed at postmortem, foil wrapped, and stored in ziplock bags at –20 °C. Samples were stored for up to 5 years before analysis; preliminary method development established no significant difference (in the number of VOCs per sample or the sum of chromatogram peak areas) between samples frozen within the same year and those frozen 8 years previously. Prior to analysis, anal glands were defrosted in a refrigerator at approximately 4 °C overnight. The complete contents of both glands were expressed manually into one 10 mL solid-phase microextraction (SPME) glass vial (Supelco), sealed, and weighed. The anal gland contents were then left to equilibrate in the vial at room temperature for approximately 1 h. The color of the secretion was categorized by comparison to a color chart, as light brown, medium brown, dark brown, mix of brown and white, or white. The smell of the secretion was categorized as fecal, sickly sweet, sweet, old oil, other unpleasant, or other not unpleasant.

Sampling of VOCs

Sample vials were placed in a water bath at 30 °C to ensure a consistent temperature during extraction. Supelco SPME fibers were used to collect VOCs eluting from samples, by exposing the fiber to the headspace of each sample for 45 min. The fiber used was StableFlex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm bonded fiber, which preliminary experiments showed to absorb far more components than other fibers tested (PDMS and polyacrylate); few components were absorbed by other fibers that were not absorbed by the DVB/CAR/PDMS. Fibers were conditioned according to manufacturer's recommendations and reconditioned for 10 min in a gas chromatography (GC) injection port at 260 °C between each sample (or for 30 min if the fiber had not been used for several h). An analysis of the fiber not exposed to any sample was conducted at least every sixth sample to detect any contamination or deterioration of the fiber, and fibers were replaced when damaged.

Analysis of VOCs

Following exposure, fibers were immediately analyzed using GC–mass spectrometry (MS) (Agilent 6890N/5973N).

Table 1 Number of otter *Lutra lutra* specimens used to investigate differences in VOCs from anal scent gland secretions

	Reproductive status (females)	Adult	Subadult	Juvenile
Male	—	27	24	13
Female	Quiescent	25	25	10
	Pregnant	9	—	—
	Lactating	25	—	—

"Quiescent" refers to females that are sexually mature but not currently pregnant or lactating.

Fibers were injected manually and desorped for 2 min at 260 °C in the split/splitless injection port fitted with an SPME liner (Supelco) in splitless mode. Samples were analyzed on a 30 m, 0.25 mm ID, 0.25 μm Zebron 5 ms capillary column (Phenomenex) with helium as carrier gas at constant pressure (13.4 psi). The oven was held initially at 40 °C for 2.5 min, then heated at 1.5 °C/min to 100 °C, followed by heating at 20 °C/min to 300 °C, and held at 300 °C for 4 min, resulting in a total programme time of 56.5 min. Temperature of transfer line, ion source, and analyzer of MS was set to 310, 230 and 150 °C, respectively. Mass spectra were recorded without solvent delay from *m*/*z* 35 to 550.

After every 4–6 samples, 0.2 μL of an external hydrocarbon standard (MA EPH Aliphatic Hydrocarbon Standard [Restek] diluted 1:50 with *n*-hexane [Fisher Scientific AnalR]) was injected using an automatic liquid injector to check the performance of the GC–MS and for calculation of retention indices. This allows standardization of retention times. Compounds were provisionally identified (minimum match factor between the deconvoluted component and the library spectra of 80%) and quantified using AMDIS (Automated Mass Spectral Deconvolution and Identification System) version 2.65 and the National Institute of Standards and Technology (NIST) Mass Spectral Library Version 2.0 (2005). Both mass spectral data and retention indices were used by AMDIS in identification. The identity of several compounds (see Table 3) was confirmed by comparison to reference standards (supplied by Sigma-Aldrich) analyzed under identical conditions. The main aim of this research was to identify differences between groups, so positive identification of all components using reference standards was not necessary.

Preliminary analyses (not presented here) tested the reproducibility of the analytical data by analyzing 3 replicates of 5 samples. The data was analyzed using principal components analysis (PCA) and hierarchical cluster analysis. Replicates from the same gland sample clustered indicating high reproducibility of the analytical data.

Statistical analysis

Pretreatment of data

Peaks with a retention time below 2 min were not included in the analysis because signals with retention times close to the hold up time of the system are not measurable with sufficient accuracy. Pretreatment of data before statistical analysis was conducted as follows: compounds found in less than 5 samples were considered unlikely to contribute to discrimination between groups (the smallest group size, pregnant females, $n = 9$) and were therefore removed. As an internal standard was not used absolute values could not be measured; instead the relative contribution of each peak to the overall scent profile was calculated, that is, data were normalized. Zero values were replaced with half the value of the lowest intensity compound measured in the entire data set.

Univariate analysis

To examine differences in relative abundance of individual compounds between groups (sex, age-class, and reproductive status, see Table 1) boxplots of all 432 compounds were visually examined and 20 compounds showing the greatest differentiation in data distribution between groups were tested statistically with a Mann–Whitney test. This was considered the most appropriate approach because a multivariate test such as multivariate analysis of variance would not be valid (data violate the assumption of having more dependent variables than cases in each cell), and individual statistical testing of each compound would incur a high risk of Type 1 error.

Multivariate analysis

PCA was applied to the full data set (all compounds, following pretreatment) to reduce the dimensionality of the data, using a correlation matrix to standardize across compounds. The resulting principal component (PC) scores were used in discriminant function analysis (DFA). DFA was used to investigate whether variation in VOCs can be used to classify the scent samples by the age, sex, or female reproductive status of the otter from which they were sourced. PC2 was log transformed in order to normalize the data, following addition of a constant (1.5) to make all scores >0. Prior probabilities were computed from group sizes. Subset validation was employed to validate the DFA; 70% of samples were used to create the model and 30% were used to test it. PCA and DFA were performed using SPSS version 16.0.

Results

Clear differences between samples were apparent to a human observer in both color and odor of juvenile and adult samples. Adult samples tended to be darker than juvenile samples (64% of adult samples and 0% of juvenile samples were dark or medium brown, whereas 57% of juvenile samples and only 1% of adult samples were white). Subadult samples were intermediate between these extremes, with 42% dark or medium brown and 24% white (Figure 1a). Adult samples typically smelt like old oil or were sweet smelling (76% of adults, 0% of juveniles), whereas juvenile samples typically smelt fecal (59% of juveniles, 3% of adults). Again, subadult samples were intermediate, with 37% old oil or sweet and 22% fecal (Figure 1b).

Complexity of chemical profiles varied considerably; the number of compounds per sample ranged from 36 to 165 (mean 112 ± 25.34) (e.g., chromatograms, see Figure 2). There was no significant difference between the 8 groups in profile complexity, that is, the number of compounds detected per sample ($\chi^2 = 10.536$, $P = 0.160$, Kruskal–Wallis) (Figure 3). Across all samples a total of 432 compounds were found of which 268 were provisionally identified using NIST library data. These comprised a complex mixture of small

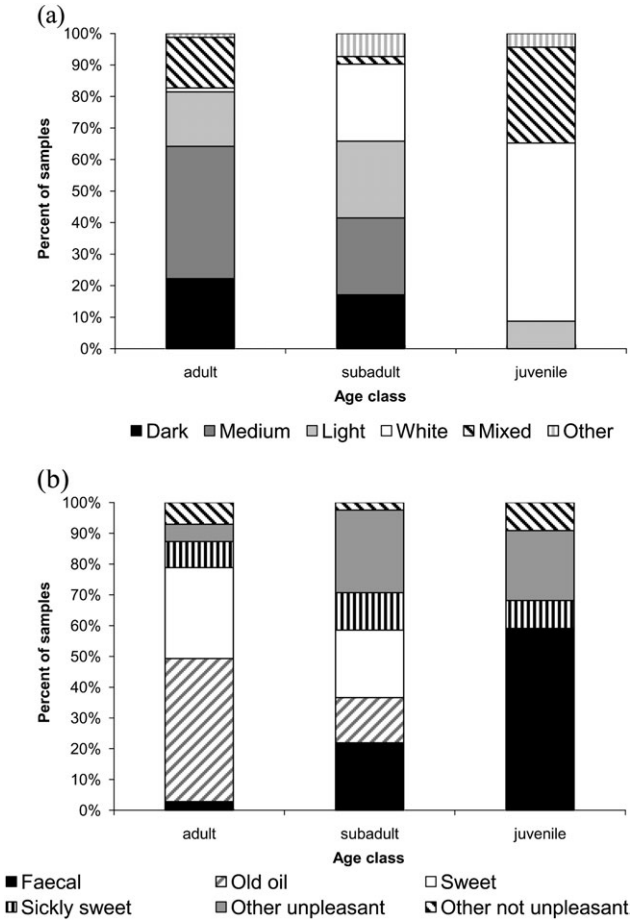

Figure 1 Human perception of **(a)** color and **(b)** scent of material expressed from otter anal scent glands.

organic acids (C3–C6), their esters, alkanes (C7–C16), alkanols (C5–C8), aldehydes and ketones (C4–C12), aromatic compounds, furanes and nitrogen, and sulfur-containing compounds, for example, pyrazines, thiols, and sulphides (listed in Supplementary Appendix 1). Typically the largest peak, and common to all samples, was provisionally identified as benzaldehyde (identity confirmed by comparison to reference standard).

Discrimination between groups

Univariate analyses

Single components provided some discrimination between age-groups but did not enable discrimination by sex or reproductive status. Two peaks differed significantly between adults and juveniles, which were identified (by comparison to reference standards) as indole (mean adults = 0.00 ± 0.01, mean juveniles = 0.11 ± 0.06; $z = -6.876$, $P < 0.001$; Mann–Whitney U test) and 2-pentylfuran (mean adults = 0.15 ± 0.10, mean juveniles = 0.01 ± 0.16; $z = -6.614$, $P < 0.001$; Mann–Whitney U test).

Multivariate analyses

Seven PCs, explaining 30% of the total variance of the 432 compounds found, were used in further analysis. DFA using all 7 PCs correctly cross-classified only 39% of samples to the 8 groups; adult male, adult female (quiescent), subadult male or female, juvenile male or female, pregnant female or lactating female (Table 2). However, within this, no pregnant or lactating females were ever misclassified as male or juvenile. PC1 scores alone clearly separate pregnant and lactating females from juveniles (Figure 4).

DFA of just the age-groups correctly cross-classified 61% of samples but no adults from the subset were misclassified as juveniles, and only 1 juvenile was misclassified as an adult. Subadult samples were often misclassified as adult or juvenile (Figure 5). Overall, 66% of samples were correctly cross-classified to their sex group, with females (74%) much better classified than males (50%). When adult data were analyzed separately, and pregnant and lactating females were excluded, sex classification was better, with 70% of males and 78% of females correctly cross-classified. Juvenile sex classification was also improved by analysis within age-group, but this was not significant and the sample size for subset validation ($n = 4$) was very low.

No significant differences were found between correctly and incorrectly classified samples in sample weight, number of compounds in each sample or slight methodological variations (e.g., different vials used for collection). This suggests the method is robust to slight variations.

Two PCs were commonly important in the discrimination of age-group, sex, and adult sex. PC1 and PC4 were the discriminating variables with the highest pooled within-group correlations with the first discriminant function for age-group (PC1 = –0.728, PC4 = 0.251 tied with PC 2 = 0.251), sex (PC1 = 0.592, PC4 = –0.643, next highest PC5 = 0.158), and adult sex DFAs (PC1 = 0.375, PC4 = –0.311, next highest PC3 = 0.222). Several furans loaded heavily onto PC1 and isomers of 2-octene loaded heavily onto PC4 (for compounds that load heavily on PC1 and PC4, see Table 3). PC3 is made up almost entirely of butanoic acid esters. The largest peak that loaded heavily on PC1 was 2-pentylfuran, which alone allows significant differentiation between adults and juveniles.

Discussion

The color and human perception of the smell of anal gland secretion differed between adult and juvenile otters but not between the sexes. The lack of color differentiation between otter sexes differs from observations recorded for other species such as beavers (Schulte et al. 1995) and badgers (Buesching, Newman, et al. 2002), where clear color

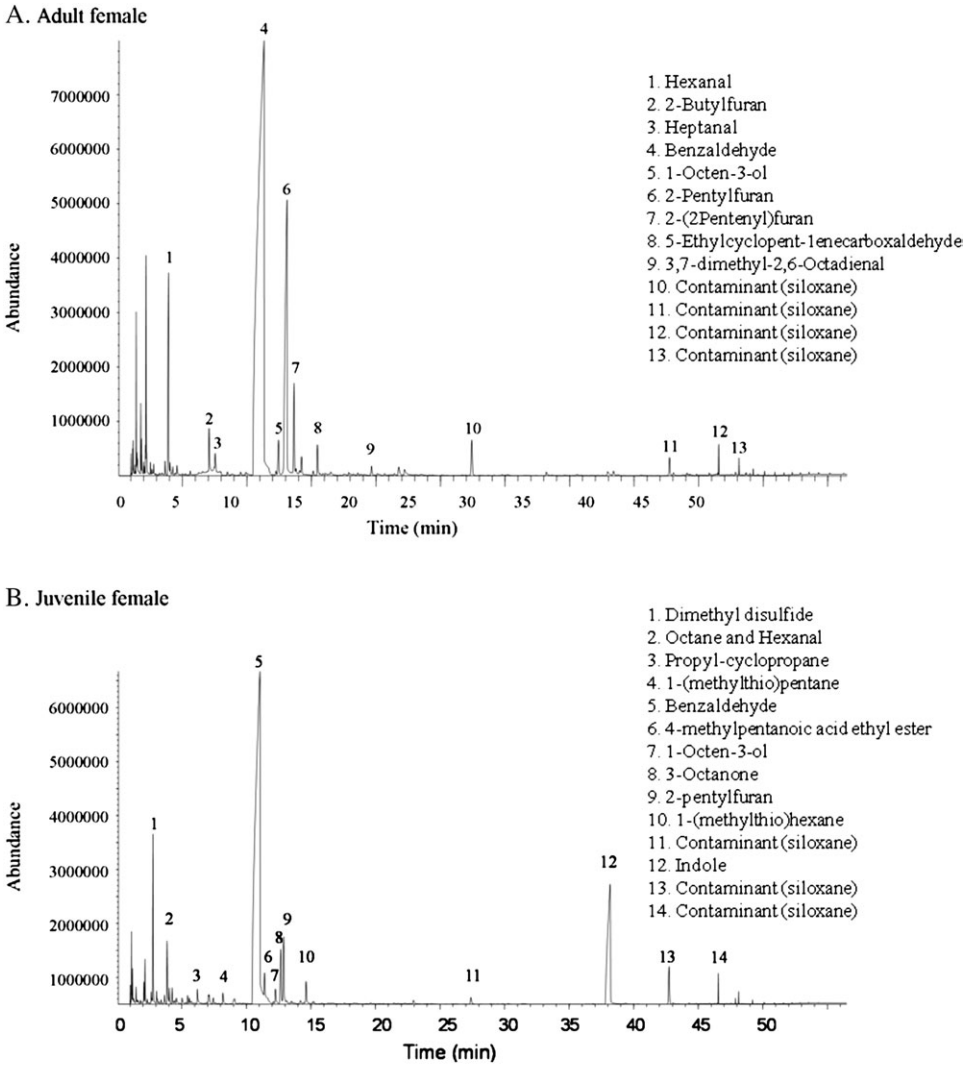

Figure 2 Example TICs (total ion chromatograms) from GC–MS analysis of VOCs from anal gland contents of an adult female **(A)** and a juvenile female **(B)** otter. Provisional identifications of the largest peaks are given, however, there are many more compounds at lower abundances.

differences in scent gland secretions are recorded. Several of the compounds involved in discrimination have been found in other mustelid scent secretions; 2-pentylfuran and 3-octanone in *Gulo gulo* (Wood et al. 2005) and both hexanal and heptanal in *M. furo* (Zhang et al. 2005). Indole is seemingly ubiquitous in the scent secretions of mustelid species; *Mustela putorius*, *Mustela nivalis*, *Mustela erminea* (Brinck et al. 1983), *G. gulo* (Wood et al. 2005), *Mustela vison* (Brinck et al. 1978; Zhang, Zhang, et al. 2002), *M. furo* (Crump 1980; Brinck et al. 1983; Zhang et al. 2005), *M. eversmanni,* and *M. sibirica* (Zhang et al. 2003). Indole and benzaldehyde (the largest compound in most samples and also found in several other mustelid species; Wood et al. 2005; Zhang et al. 2005; Brinck et al. 1983) were previously thought to be absent from otter scent (Brinck et al. 1983), exemplifying how employment of different analytical methods can improve results. Isomers of 2-octene are previously unreported in mustelid scent secretions, although they do occur in human faeces (Garner et al. 2007) and were found here to load heavily on PC4, important in discrimination between groups. Although the methods used here reveal several compounds "new" to mustelid or otter scent, it is recognized that these methods may also have limitations and not sample all VOCs from otter scent.

Age differences

Adult and juvenile otter anal gland VOCs differ in the relative abundance of indole and 2-pentylfuran; more complex multivariate differences also occur (as demonstrated with PCA and DFA) even though only one of these compounds (2-pentylfuran) loaded heavily onto the PCs used in DFA. Although only 61% of samples were classified correctly to their age-group following subset classification, there was only one

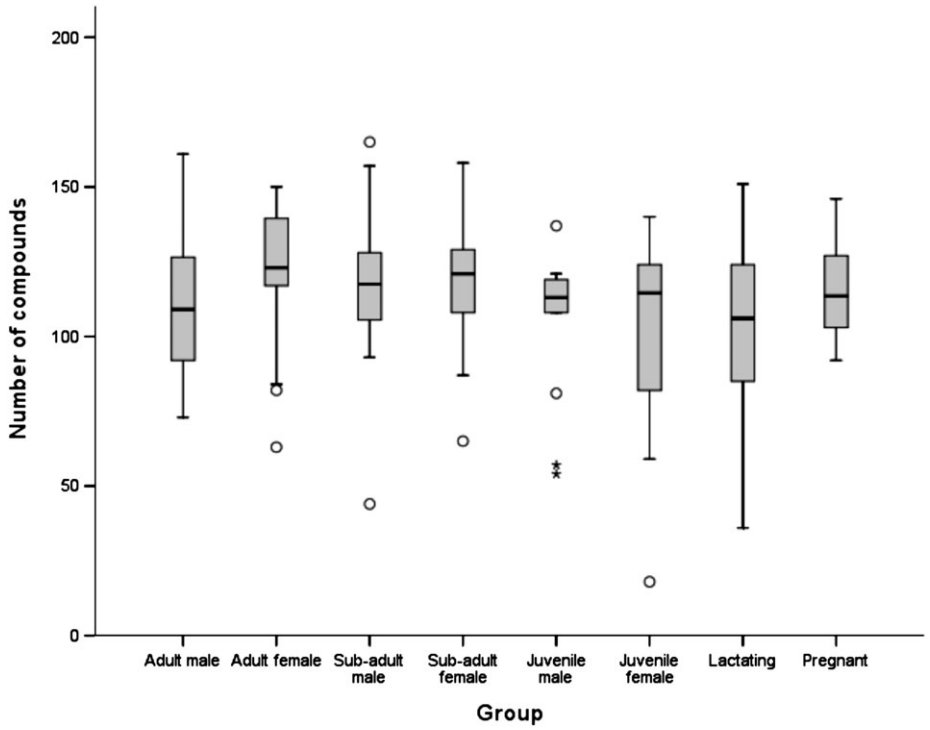

Figure 3 Number of compounds detected in samples from 8 different age, sex, and reproductive status groups of otter. A Kruskal–Wallis test showed no significant difference in number of compounds between the 8 groups (χ^2 = 10.536, P = 0.160).

Table 2 Results of DFA subset validation of otter anal scent gland contents

	% Of samples correctly classified	Discriminant function	Wilk's λ	χ^2	P
Eight groups	39.0 (no pregnant or lactating as male or juvenile)	1	0.201	170.940	<0.001*
		2	0.604	53.783	0.029*
		3	0.740	32.086	0.156
		4	0.855	16.684	0.406
		5	0.927	8.021	0.532
		6	0.988	1.244	0.871
		7	0.999	0.055	0.814
Three age-groups	61.0 (no adults as juveniles and 1 juvenile as adult)	1	0.417	95.236	<0.001*
		2	0.917	9.483	0.148
Sex	65.9 (female 74.1%, male 50%)	1	0.715	36.678	<0.001*
Adult male and female (excluding pregnant and lactating females)	73.7 (female 78%, male 70%)	1	0.572	14.781	0.039*
Subadult male and female	61.1	1	0.581	13.826	0.054
Juvenile male and female	100	1	0.465	9.565	0.215

The model is created using 70% of samples and 30% are tested with it. Wilk's λ is a measure of how well each function separates groups. Smaller values indicate a greater discriminatory power of the function. The associated chi-squared value tests the hypothesis that the means of the functions are equal across the groups. Significant values indicate that the discriminant function does better than chance at separating the groups.
Significant discriminant functions: *P < 0.05, **P < 0.01, ***P < 0.001.

misclassification between adults and juveniles. Age is, of course, continuous, and there is currently no accurate method for ageing otters (Sherrard-Smith and Chadwick 2010). Otters used in this study were assigned to the given age-groups (adult, subadult, and juvenile) at postmortem, based on body size and reproductive status (Chadwick 2007), which because of

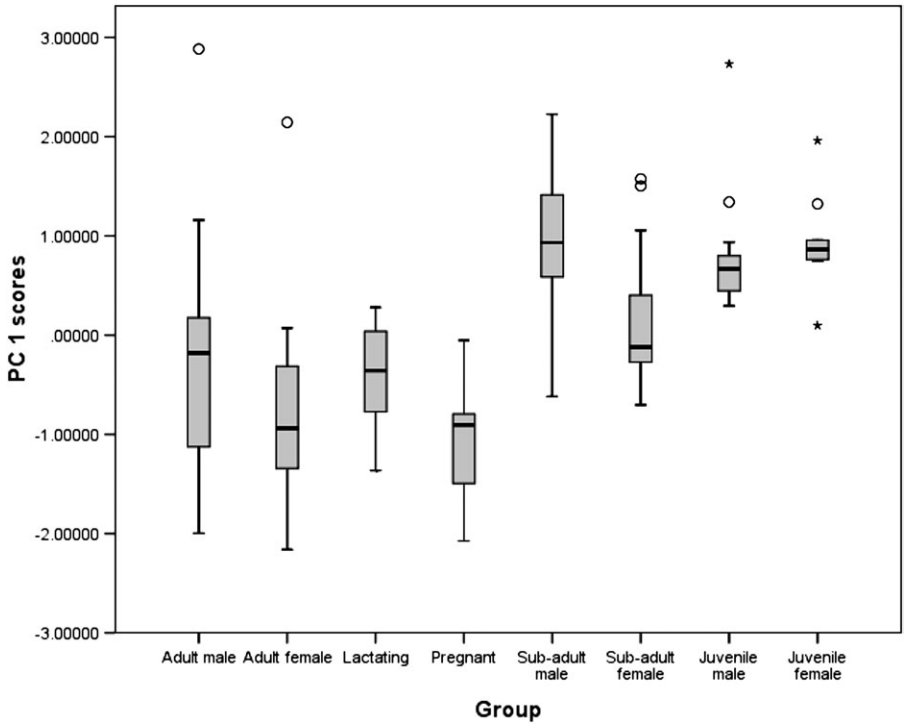

Figure 4 Difference in PC1 value from chemical profile of anal gland secretion between the 8 different otter groups investigated. Statistical discrimination was performed using 7 PCs (test statistics are presented in Table 3), here only PC1 is plotted for illustrative purposes.

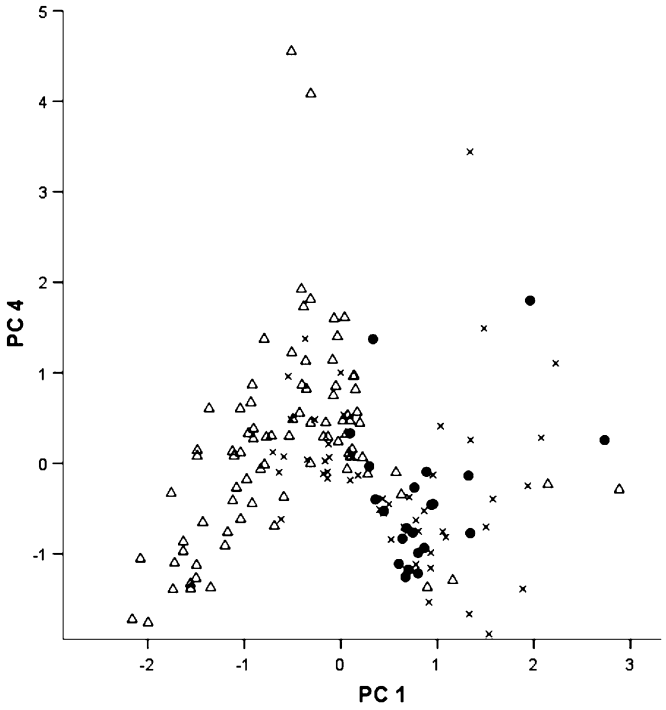

Figure 5 PC1 and PC4 from chemical profiles of otter anal gland secretion showing some differentiation between age-groups (△ adult, x subadult, and ● juvenile). Statistical discrimination was performed using 7 PCs (test statistics are presented in Table 3), PC1 and PC4 are plotted here as they were most important in the DFA.

differences in development rates, may not always accurately reflect actual age, leading to unavoidable error in assignment. Although there is some misclassification of scent profiles between adults and subadults, as well as between juveniles and subadults, we can successfully discriminate between adults and juveniles. In a review of mammalian social odors, Brown (1979) found little chemical evidence for age specific odors. Since then, however, age differences in chemical composition of scent have been found in some species (Buesching, Waterhouse, et al. 2002; Osada et al. 2003; Schaefer et al. 2010). In the current study, differences in scent between juvenile and adult anal gland samples were apparent even to the human nose; juvenile samples were much stronger and fecal smelling. Indole, which was present in all juvenile samples (usually at high relative abundance) but found in very few adults, does have an unpleasant odor at high concentrations (Lewis 2007). Adult otter anal gland samples were more frequently sweet smelling and spraint is commonly described by field workers as smelling of jasmine or freshly cut hay.

The spraints of otter cubs are usually larger than adult spraints and adult male spraints are usually smaller than those of females (Kruuk 2006). This could be because adult males deposit spraint more frequently for communication purposes, whereas cubs simply spraint for fecal elimination. The distinct difference in scent of juveniles may simply be an immature body function or as a result of dietary differences, rather than functional signaling, as it is likely that many of the otters categorized as juveniles in the present study were not fully weaned

Table 3 PCA loadings (with values greater than ±0.7) of PC1 and PC4 from volatile analysis of *Lutra lutra* anal gland secretions

	Retention index	Occurrence (number of samples)	Mean relative abundance (% area) ± standard deviation
PC1			
2-Propylfuran	834	48	0.30 ± 0.17
Hexanal[a]	837	141	4.81 ± 4.78
Unknown 87	838	89	0.04 ± 0.04
2 Butylfuran	890	124	0.61 ± 0.61
Heptanal[a]	898	128	0.26 ± 0.26
Unknown 85	914	90	0.07 ± 0.06
Unknown 78	920	42	0.06 ± 0.05
2-Pentylfuran[a]	986	156	10.25 ± 9.61
3-Octanone[a]	991	145	1.86 ± 2.89
2-(2-Pentenyl)furan	997	110	2.24 ± 2.00
Octanal[a]	1000	44	0.14 ± 0.09
3-Pentylfuran	1003	66	0.04 ± 0.02
5-Ethylcyclopent-1-enecarboxaldehyde	1020	114	0.85 ± 0.88
Unknown 86	1076	77	0.04 ± 0.03
2-Hexylfuran	1082	64	0.07 ± 0.05
Potential triene C13	1314	72	0.22 ± 0.14
Potential diene C13	1319	61	0.06 ± 0.04
PC4			
(*E*)-2-Octene[a]	840	157	0.66 ± 0.75
(*Z*)-2-Octene	844	125	0.63 ± 0.56
Unknown 66	847	149	0.17 ± 0.17
Potential C11H18 isomer	1070	44	0.25 ± 0.20
Cyclooctanemethanol	1073	118	0.07 ± 0.07
Potential C11H18	1078	121	0.23 ± 0.24
2,9-Undecadiene	1102	140	0.23 ± 0.25

[a]Compound identity confirmed by comparison to reference standards.

(based on morphometric data, "juveniles" were thought to be <20 weeks; otters are weaned at approximately 14 weeks; Harris and Yalden 2008). There is no firm evidence of the age at which otter cubs deposit spraint as a marking behavior rather than for fecal elimination. Scent marking is characterized by repeated deposition of small amounts of material at selected sites (Kleiman 1966), whereas deposition purely for fecal elimination tends to be more voluminous and has no pattern of deposition. Erlinge (1968) mentioned 2 captive cubs displaying marking behavior when they were first observed at around 8–9 months, but observations were not made prior to this and so this behavior may have developed at a younger age. Other observations of juvenile otter sprainting behavior have been made at around 5 months of age (Polotti et al. 1995; Green 2000), so the otters used in the present study may not have been scent marking.

Sex differences

Sex differences in VOCs were found between adult male and female otters but not in younger otters, suggesting a role in mate attraction. The nature of sex differences in scent varies between species. Differences in the relative abundance (analog coding) and presence/absence (digital coding) of individual compounds have both been found in mustelids (Zhang et al. 2003, 2005). Multivariate analyses combine analog and digital coding and have been used to reveal subtle signaling differences between organisms involving complex

mixtures of compounds, for example, results presented in this article and in humans (Penn et al. 2007).

Despite chemical communication of sex for the purpose of mate attraction being almost omnipresent (Johansson and Jones 2007), an absence of sex differences in scent marks has been found in some mustelids, for example, mink, *M. vison* (Brinck et al. 1978; Zhang, Zhang, et al. 2002). The apparent disparity between species in communication of sex through scent marks may reflect differences in ecology or limitations in the techniques used to analyze the scent marks either statistically (as described above) or chemically (e.g., the extraction method used or detection limits of GC–MS). A larger sample size combined with different methods of chemical and statistical analysis in our study allowed us to exceed previous findings by Bradshaw et al. (2001) and to discriminate between adult male and female otters. Similarly, the use of different sampling techniques improved VOC analysis from 2 other mustelid species (Zhang et al. 2003). Analysis of different scent secretions from the same species may also reveal differences that were previously thought to be absent, for example, sex differences were found in badger subcaudal gland (Buesching, Waterhouse, et al. 2002) but not in anal gland secretions (Davies et al. 1988). Otters do not have subcaudal glands, but it is possible that some messages are communicated in nonvolatile components of otter scent, as is well documented in mice (Hurst et al. 2001; Nevison et al. 2003); analysis of nonvolatiles may reveal simpler differences between otter sexes.

Female reproductive status

The clearest differences between the 8 groups of otters using DFA were between pregnant or lactating females and male or juvenile otters. Dietary or hormonal differences may explain the differences between pregnant or lactating females and juvenile otters. Reproductive status also affects the scent of female badgers (Buesching, Waterhouse, et al. 2002). Parental care and therefore the recognition of young are an important factor in the evolution of social odors in mammals and partly explain scent differences between individuals (Brennan and Kendrick 2006). Generalized scent signals of pregnancy or lactation also exist, for example, nipple-search pheromone in humans and other mammals (Porter and Winberg 1999) or the generalized attraction to nest odors in gerbils (Gerling and Yahr 1982). Female otters are said to be very secretive when they are pregnant or lactating and natal are dens hard to find (Kruuk 2006). Signaling their reproductive status to males is likely to be disadvantageous, as males are known to commit infanticide (Simpson and Coxon 2000). Pregnant females or those with very young cubs are said to spraint in water (Jenkins and Burrows 1980; Kruuk 2006), and this might help to hide the scent differences shown in the current study.

In summary, our results indicate simple age differences in the VOCs from anal scent gland secretion of Eurasian otter *L. lutra* and more complex multivariate differences with sex and reproductive status of adult otters. These results are a first step toward further understanding of otter scent communication, which might be used in the future to help monitor wild populations of this species.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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