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Brown bears possess anal sacs and secretions may code for sex

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Abstract

Olfactory communication occurs in carnivores and many scent-mark with anal gland secretions (AGS), which contain a variety of information including sex-related cues. Currently, there is disagreement about whether bear species, other than the giant panda *Ailuropoda melanoleuca*, possess anal glands or anal sacs. We documented anal sacs in brown bears *Ursus arctos* and analyzed AGS from 17 free-ranging, sexually mature individuals using gas chromatography–mass spectrometry. We hypothesized that brown bear AGS codes for sex, as it does in giant pandas, and predicted that AGS shows sex differences in gas chromatogram (GC) profiles, number of compounds, the digital and analog coding of chemical compounds, and color. We found 90 different compounds. Our results support the predictions that male and female AGS differs in GC, analog coding and possibly color. However, we found no significant difference between sexes in number of detected compounds or in the digital coding. Our results confirm that brown bears possess anal sacs, that secretions likely relay information about sex, and suggest other chemical information critical to the bears' social system is encoded in the AGS.

Introduction

Olfactory communication plays an important role in mammalian social and reproductive behavior, and many species use pheromones to send information about individual identity, reproductive status, territory boundaries and kin relations (Wyatt, 2003; Müller-Schwarze, 2006). Pheromones are often present in urine, various scent glands and feces and contain a mixture of chemical compounds with varying volatility (Brown & Macdonald, 1985; Wyatt, 2003).

Glandular tissues in the anal region of mammals are referred to collectively as 'anal glands' (Macdonald, 1985). Anal sacs are not anal glands (Scott, Miller Jr & Griffin, 2001), but secretion from the sacs is produced by glands in the anal region and is called anal gland secretion (AGS). There can be species differences in tissue structure, location and function as well as postural and behavioral deposits of AGS. In species of Hyanidae, Felidae and some Mustelidae, AGS is deposited separately from feces (see Macdonald, 1985). The use of AGS in olfactory communication has been documented in carnivores, as a code for sex in some mustelids (*Mustela* spp.) (e.g. Zhang *et al*., 2002, 2003, 2005), for sex (Yuan *et al*., 2004; Zhang *et al*., 2008), individuality (Hagey & Macdonald, 2003; Zhang *et al*., 2008) and age (Hagey & Macdonald, 2003) in the giant panda *Ailuropoda melanoleuca*, and for social status and individual identity in the spotted hyena *Crocuta crocuta* (Burgener *et al*., 2009). The AGS color differs between sexes in the Eurasian beaver *Castor fiber* (Rosell & Sun, 1999) and the subcaudal gland of European badgers *Meles meles* (Buesching, Newman & Macdonald, 2002*a*). In European badgers, there is a sex-related chemical difference in subcaudal glands (Buesching, Waterhouse & Macdonald, 2002*b*), but not anal sacs (Davies, Lachno & Roper, 1988).

Few studies have investigated AGS in large carnivores, such as bears (Ursidae). Giant pandas possess large anal glands that secrete a waxy substance (Schaller *et al*., 1985). There is, however, virtually no information on AGS in the other seven bear species (Breiter, 2008) and there is disagreement about whether they even possess anal sacs. Pocock (1921) found greatly reduced anal sacs in the American black bear *Ursus americanus*, but did not find them in the brown bear *Ursus arctos*. Schaffer (1940) stated that the brown bear has anal glands, whereas Dyce, Sack & Wensing (1996) stated that bears do not.

One reason why little is known about olfactory communication in bears may be that they are elusive, have large home ranges (our study area: male, 1055 km^2; female, 217 km^2) (Dahle & Swenson, 2003), and are difficult to capture (Dahle & Swenson, 2003; Arnemo *et al.*, 2006). The brown bear is a solitary species with a polygamous mating system (Schwartz, Miller & Haroldson, 2003; Bellemain *et al.*, 2006). Males reach sexual maturity as early as 3 years of age (Zedrosser *et al.*, 2007), and primiparity varies from age 4 to 6 (McLellan, 1994; Zedrosser *et al.*, 2009). There is evidence that free-ranging brown bears can recognize and/or discriminate between individuals, because related females show more home-range overlap than unrelated females; suggesting that related females tolerate each other more than unrelated females (Støen *et al.*, 2005). Additionally, Zedrosser, Dahle & Swenson (2006) reported that young male brown bears disperse from their natal areas to avoid mate competition with older bears, implying that brown bears can discriminate between individuals and/or age classes (e.g. adults vs. subadults).

Thus, brown bears may use olfactory information for kin and status recognition, as in giant pandas (Swaisgood, Lindburg & Zhou, 1999), steppe polecats *Mustela eversmanii* (Zhang *et al.*, 2002), and coyotes *Canis latrans* (Tegt, 2004). Tschanz, Mayer-Holzapfel & Bachmann (1970) observed that captive subadult bears withdrew in response to adult urine and feces at rubbing sites, suggesting a social communication function. The ability to discriminate between sexes benefits the scent donor by advertising its presence or attracting a potential mate. In turn, the receiver of the scent benefits by avoiding conflicts or recognizing individuals in later encounters (i.e. scent matching) (Gosling, 1982). American black bears rubbed marking trees more frequently in the mating season (Burst & Pelton, 1983), and giant pandas increased the use of AGS during this period (Schaller *et al.*, 1985).

Because brown bears have large home ranges, they have a vast area to scent mark. These marks should be durable to be an effective form of communication. Compounds of low volatility [i.e. high molecular weight (MW)] persist in the environment longer than those of high volatility, and are therefore more conducive to long-lasting or delayed communication because infrequent renewal could save substantial energy associated with patrol (Yuan *et al.*, 2004).

The aim of this study was to determine if free-ranging brown bears possess anal sacs, and, for the first time, chemically investigate their AGS. Although several codes potentially exist in brown bear AGS, we hypothesize that the AGS codes for sex, as in giant pandas (Yuan *et al.*, 2004), and predict that AGS shows sex differences in gas chromatogram (GC) profiles, number of compounds, digital (presence/absence) and analog (relative abundance) coding of chemical compounds and color.

Materials and methods

Study area and animals

The study was conducted in Dalarna and Gävleborg counties in south-central Sweden (61°N, 14°E), within the southern part of the Scandinavian brown bear population (Sahlèn *et al.*, 2007), and with a bear density at ~30 bears/1000 km^2 (Zedrosser *et al.*, 2006). The area is forest, dominated by scots pine *Pinus sylvestris*. Common tree species are Norway spruce *Picea abies*, birches (*Betula* spp.), aspen *Populus tremula* and lodgepole pine *Pinus contorta*. Timber management for clearcutting is intensive in the area, and together with roads, bogs and lakes, creates a patchy landscape.

As part of a long-term research project, we darted and immobilized free-ranging bears from a helicopter using a remote drug delivery system (Dan-Inject®, Børkop, Denmark) with a combination of tiletamine/zolazepam and medetomidine (Arnemo *et al.*, 2006; Arnemo & Fahlman, 2008). We collected AGS samples during two periods before the mating season (18 April–18 May 2007, 6–30 April 2008; Table 1). Sex was determined by genital examination. Age of bears not captured as yearlings was determined based on cementum annuli in the root of the upper first premolar tooth (Matson *et al.*, 1993) analyzed at Matson's Laboratory (Milltown, MT, USA). Sexually immature bears (< 3 years old) were excluded from analysis.

AGS collection

Anal sacs were located and identified by direct observation and manual palpation. We manually squeezed AGS from immobilized bears lying on their side or stomach by applying pressure on each anal sac separately. Samples were collected in 40 mL glass vials with teflon-lined caps (Lab Safety Supply®, Janesville, WI, USA) and immediately put on ice. We used latex gloves during AGS collection to avoid contamination by human scent. All samples were frozen at −20 °C within 8 h of collection and kept frozen until analysis.

AGS color comparison

We compared the secretion colors with the natural color system (Scandinavian Colour Institute, Stockholm, Sweden) to evaluate sex differences in AGS color (Rosell & Sun, 1999). AGS colors were also ranked by eye into 12 categories from 1 = light to 12 = very dark (Buesching *et al.*, 2002*a*).

Chemical sample preparation

We performed a pilot study with different solvents and found that toluene–methanol 3:1 extracted most compounds from the AGS. We transferred 0.1 g of AGS into a glass test tube with a sterile needle. We added 1 mL of toluene–methanol 3:1 and vortexed the solution for 15 s. The compounds were extracted for 2 h at room temperature before centrifuging the sample for 3 min at 686 ***g***. We covered the glass test tube with aluminum foil during extraction and centrifugation to avoid loss of volatile compounds. Next, we pipetted the particle-free solution into a GC-vial and used this solution in the analyses.

Chemical analysis

We used a Hewlett-Packard (HP, Oslo, Norway) 6890 Series II gas chromatograph equipped with a non-polar HP-5 MS 5% phenyl–methyl–siloxane column (30.0 m long × 0.25 mm ID × 0.25 μm film thickness) connected to a HP 5973 Series mass spectrometer detector in the splitless mode. Helium gas was set to a constant flow of 1.0 mL min^{-1} and the injection port temperature was 270 °C. The purge flow to split vent was 49.8 mL min^{-1} at 1.00 min. The instrument was calibrated before analysis.

We injected 1 μL of the particle-free AGS solution into the gas chromatograph–mass spectrometer (GC–MS) using an auto-injection system (Agilent 7683 Series Injector, Oslo, Norway). We used a HP single taper liner (4 mm inner diameter) with glass wool, and set the solvent delay to 5 min for every run to avoid damaging the detector. Initial oven temperature was set to 55 °C for 2 min then increased 6 °C/min to 310 °C, which was maintained for 5 min. Each run lasted 49.50 min; the first 8 min were eliminated from analysis because peaks in this interval stemmed from either solvent or the column. Control samples were run before, in the middle, and after all samples to control for changes in abundance or retention time. No major changes were observed.

We tentatively identified compounds by matching the retention time and mass spectra of the GC peaks with structures of 70 000 known compounds in the Wiley 275 Library, using a computer search. Structures of unidentified compounds were added to a new library and included in the search. The new compounds could then be recognized in different samples by comparing structures and retention times. The mass spectra from the GC peaks and the library were compared visually to determine if suggestions from the computer were reasonable. A positive identification of the compounds through known standards was not conducted because it was not the focus of this study.

The area of every peak was determined by computer-aided integration. We set the threshold to 17.0 to avoid integration of peaks that resulted from background noise. In order to quantify the relative abundance of each compound, we converted the single peak area into the percentage of the total peak area of the GC.

Olfactory information can also be examined by digital and analog coding of chemical compounds in the secretion (Sun & Müller-Schwarze, 1998*a*,*b*). Digital and analog coding is commonly used with GC–MS to reveal sex differences in composition of mammal AGS (e.g. Zhang *et al.*, 2003, 2005). We encoded the tentatively identified compounds by 0 (absent) and 1 (present) for all samples, and formed two digital matrixes of *X*-variables (detected compounds) and *Y*-variables (individuals). From the matrixes we examined the number of detected compounds by individual and sex.

Statistical analyses

We analyzed the data with partial least squares regression (PLSR) (Wold, Martens & Wold, 1983, 1984; Wold, Sjöström & Eriksson, 2001). PLSR was used because it can analyze data with strongly correlated, noisy and numerous *X*-variables, and simultaneously model several response variables, *Y* (Wold *et al.*, 1983, 2001). PLS1, a type of PLSR, uses information in the *Y*-matrix to find the *Y*-relevant structure in the *X*-matrix (Esbensen, 2002) (for details see Rosell & Steifetten, 2004). As a basis for comparison, the abundance was measured for every time unit (165 time units min^{-1}) on the retention scale of the GC. The measured values formed a GC-matrix of *X*-variables (7012 time measurements) and *Y*-variables (sex of donors of 17 AGS samples). All values were scaled by mean normalization and standard normal variate to minimize the effect of variation in abundance between samples. Because of low sample size we used leverage correction to estimate the prediction residuals (Esbensen, 2002). The PLSR method extracts a small number of PLS components (PCs), which represent the relevant latent dimensions of the model. We used the values of validated R^2 and root mean square error of prediction (RMSEP) to evaluate the results. RMSEP is a measurement of the average difference between predicted and measured response values, with 0 showing least difference. We used the statistical software The UNSCRAMBLER 9.7 (Camo Software AS, Oslo, Norway).

We analyzed sex differences in the number of detected compounds with the Mann–Whitney *U*-test (Zar, 1998). A compound was defined to be sex specific if it was found in all males or females (Andersen & Vulpius, 1999). To check for sex difference in the digital composition of AGS, the digital matrices were placed in a hierarchical cluster analysis with squared Euclidean distance (e.g. Yuan *et al.*, 2004). We used cluster analysis because the matrices had more variables (compounds) than observations (individuals), and canonical discriminant analysis was therefore unsuitable (Johnson & Wichern, 1992). We checked for sex differences in analog coding of AGS using relative abundance of each compound in quantitative analyses. The Mann–Whitney *U*-test (SPSS for Windows, version 15.0, SPSS Inc., Chicago, IL, USA, 1999) was used to investigate the difference between the sexes in relative abundance and color. Significance levels were set to $P < 0.05$, and we defined a *P*-value of 0.05–0.1 as marginally significant.

Results

Anal sac description

The topography of the two anal glands is similar to that of the domestic dog *Canis familiaris*; the duct from each gland opens laterally at the cutaneous zone of the anal canal. All captured brown bears possessed paired anal sacs (Fig. 1). Males ranged in age from 3 to 17 years and females from 3 to 18 years (Table 1). The AGS had a clay-like substance with an unpleasant odor, but we detected no obvious sex difference in odor. AGS color varied from nearly black to light gray (Table 2). An overlap in color existed, but AGS of males was significantly darker than that of females ($U = 7.5$, $n_{male} = 5$, $n_{female} = 11$, $P = 0.02$).

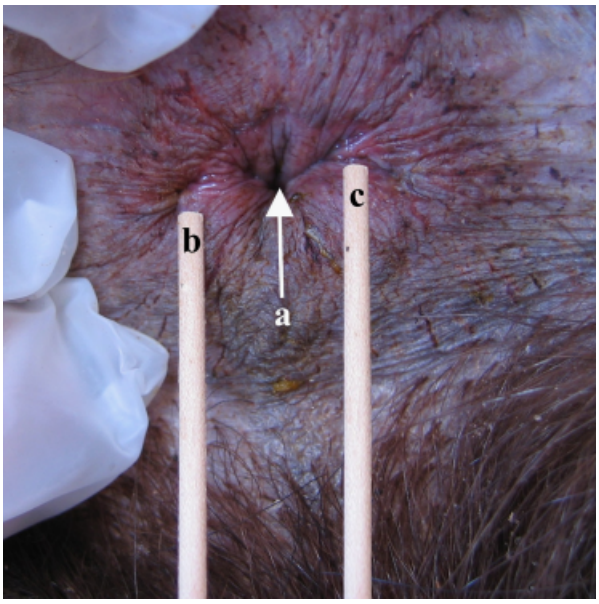

Figure 1 The anal region of a brown bear *Ursus arctos* showing the anus (a) and the location of the left (b) and right (c) opening of the anal sacs, indicated by the wooden sticks (photo: Andreas Zedrosser).

Table 1 Individual number, sex and age of brown bear *Ursus arctos* donors of anal gland secretion

Number	Individuals	Date collected	Sex	Age
1	W9101[a]	25 April 2008	F	18
2	W9301	18 April 2007	M	17
3	W0236[a]	28 April 2008	F	15
6	W0624	21 April 2007	F	12–15[b]
7	W9403[c]	04 May 2007	F	14
8	W0004[a]	24 May 2008	F	13
9	W9903[a]	30 April 2008	F	11
10	W0424[a]	30 April 2008	M	11
12	W0717	18 May 2007	M	>10[b]
13	W0803	14 April 2008	M	>10[b]
14	W0626	03 May 2007	F	8
18	W0217	03 May 2007	F	6
19	W0517	20 April 2007	F	6
23	W0416	22 April 2007	F	4
24	W0415	20 April 2007	F	4
26	W0508[a]	29 April 2008	F	3
28	W0612	06 April 2008	M	3

[a]These individuals were not used in analysis of total number of compounds, digital or analog coding because some data were lost.
[b]Exact age of these individuals could not be determined.
[c]This individual was not used in the color comparison.
M = male, F = female.

GC comparison

We found a difference in GCs between male ($n = 5$) and female ($n = 12$) AGS (Fig. 2). The PLS1 score plots tended to separate male and female AGS (Fig. 2). R^2 was 0.82, indicating a high predictive ability of the model. The RMSEP was 0.20, indicating a small average difference between predicted and measured response values. Of the total variation within the AGS GCs, PC1 explained 39% of the *X*-variance and 30% of the *Y*-variance, whereas PC2 explained 20% of the *X*-variance and 38% of the *Y*-variance.

Digital coding

We tentatively identified 90 compounds in the AGS that we were able to analyze ($n_{male} = 4$, $n_{female} = 7$; Tables 1 and 3). Unfortunately, some data were lost and no secretion remained to repeat GC–MS analysis, resulting in a lower sample size for total number of compounds, and digital and analog coding. The samples we analyzed are identified in Table 1. In general, these compounds were classified as fatty acids, hydrocarbons and steroids. Of the compounds with determined MW, 68% were above 300 MW (Table 3). We found a total of 74 (mean $\pm$ SD $= 38.00 \pm 8.87$) different compounds in males and 59 (29.14 ± 7.78) compounds in females (Table 3), but there was no sex difference ($U = 6.5$, $n_{male} = 4$, $n_{female} = 7$, $P = 0.12$) nor sex-specific compounds. Typical GCs of female and male AGS is shown in Fig. 3a and b. Hierarchical cluster analysis did not show any clear digital classification patterns in AGS between the sexes (Fig. 4).

Analog coding

We found differences between male and female AGS in the relative abundance of five of the shared compounds (Table 3). Females had a significantly higher abundance of four compounds than males: no. 57, 60, 73 and 80 (all steroids; $U = 2$, $P = 0.023$; $U = 3$, $P = 0.038$; $U = 1$, $P = 0.014$; $U = 0$, $P = 0.008$, respectively; Fig. 5), and males had a marginally significantly higher abundance of compound no. 76 (a steroid; $U = 4$, $P = 0.058$; Fig. 5).

Discussion

Our study is the first to confirm conclusively that brown bears possess anal sacs, and to investigate chemically their secretions. Our results supported our predictions that male and female AGS differ in GC, analog coding, and color, suggesting that AGS likely codes for sex in brown bears. However, our predictions of sex differences in number of detected compounds and digital coding of AGS were not supported.

Brown bear anal sacs were similar to those of domestic dogs, which are located between the external and internal sphincter muscles and help empty the contents of the intestine (Dyce *et al.*, 1996). It is unknown, but likely, that brown bears are capable of excreting AGS independently of feces, as observed in other carnivores with similar anal sacs, such as wolves *Canis lupus* (Asa *et al.*, 1985).

It is probable that brown bear AGS mediates information pertaining to mating. The amount of extractable AGS seemed to decrease as the mating season progressed

Table 2 Color of the anal gland secretions of brown bears (*Ursus arctos*)

Individual	Sex	Age	Color	Color rank[a]	NCS-code[b]
W9301	M	17		12	S8505-Y80R
W0424	M	11			
W0612	M	3		11	S8010-Y50R
W0508	F	3			
W0217	F	6		10	S8010-Y30R
W0004	F	13		9	S8005-Y50R
W0717	M	>10		8	S7020-Y30R
W0803	M	>10		7	S7020-Y20R
W0415	F	4			
W0624	F	12-15		6	S7010-Y30R
W0416	F	4		5	S6020-Y30R
W9101	F	18		4	S5020-Y20R
W9903	F	11			
W0626	F	8		3	S5010-Y30R
W0517	F	6		2	S5010-Y10R
W0236	F	15		1	S4040-Y20R

[a]The colors were ranked by eye in 12 categories (from 1 = light, to 12 = very dark).
[b]Natural color system (NCS), Scandinavian Colour Institute AB.

(S. Brunberg, pers. comm.), but the production rate of AGS throughout the year is unknown. Less AGS later in the mating season implies that the normally solitary bears use AGS more frequently to communicate their presence when they are more likely to meet, as in giant pandas (Schaller *et al.*, 1985). On the other hand, a lower production of secretion after the mating season, as in the subcaudal gland of European badgers (Buesching *et al.*, 2002*a*), is also possible.

We documented a sex difference in AGS color; male secretion averaged darker than female secretion. In contrast to North American beavers (Schulte, Müller-Schwarze & Sun, 1995), aardwolves (Sliwa, 1996), Eurasian beavers (Rosell & Sun, 1999) and European badgers (Buesching *et al.*, 2002*a*), the color of male and female AGS overlapped in brown bears. Therefore, AGS color should not be used as the sole source for determining sex.

The sex differences in GCs and in analog coding imply that sex identification might be coded through a specific mix of several compounds (Albone, 1984), or by the relative abundance of some compounds. The total number of compounds detected in brown bear AGS is relatively high compared with other scent-marking carnivores, like steppe polecat *Mustela eversmanni* (17 compounds) (Zhang *et al.*, 2003), Siberian weasel *Mustela sibirica* (14 compounds) (Zhang *et al.*, 2003) and domestic dog (13 compounds) (Preti *et al.*, 1976). However, the total number of compounds is similar to giant panda AGS (95 compounds) (Yuan *et al.*, 2004). Many of the compounds we identified in brown bear AGS were fatty acids, fatty acid esters, steroids and hydrocarbons, which are also found in wolves *C. lupus* (Raymer *et al.*, 1985), domestic dogs (Natynczuk, Bradshaw & Macdonald, 1989), giant pandas (Yuan *et al.*, 2004) and wolverines *Gulo gulo* (Wood, Terwillinger & Copeland, 2005). Similar to our results, Yuan *et al.* (2004) also found analog coding for nine compounds in giant panda AGS. The major differences in relative abundance of shared compounds in the giant panda were found in four steroids, but we cannot confirm whether these are the same four steroids as in brown bears.

We did not find sex-specific compounds in the AGS. Yuan *et al.* (2004) also concluded that information about

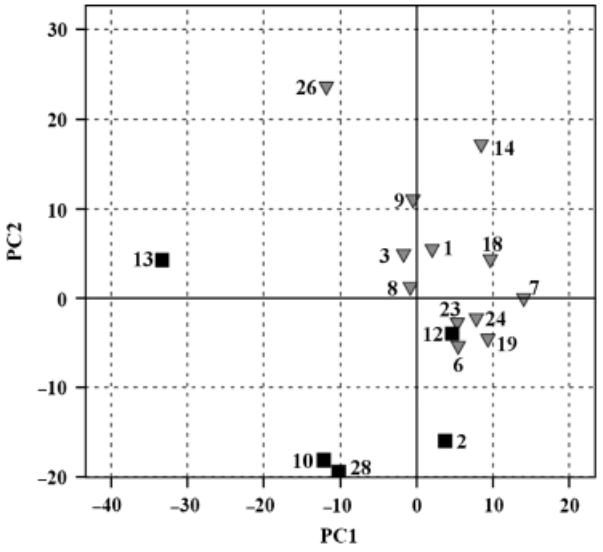

Figure 2 Partial least square regression score plot showing the position of each gas chromatogram of brown bear *Ursus arctos* anal gland secretions [■: male ($n=5$); ▽: female ($n=12$)] of the two first components, PLS component (PC) 1 and PC2. The numbers in the plots correspond with the number in Table 1.

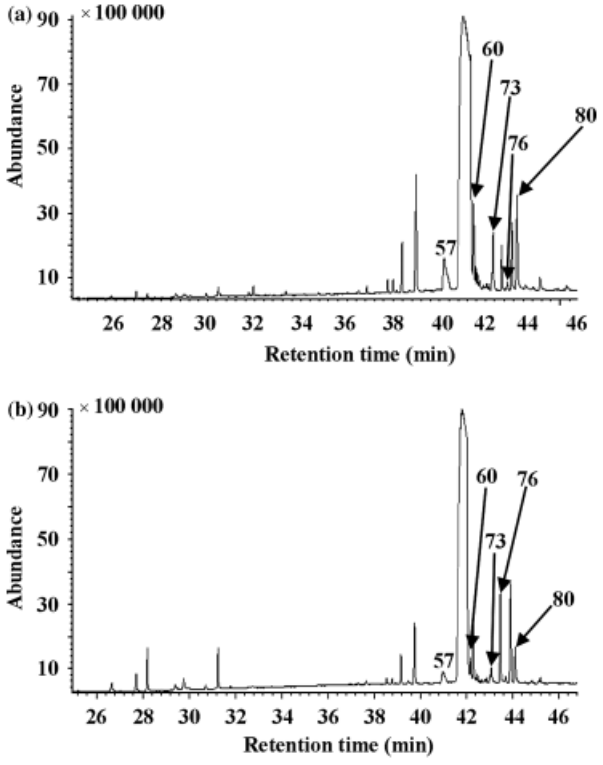

Figure 3 Typical gas chromatograms (GC) of the anal gland secretion from a female (a) and male (b) brown bear *Ursus arctos*. The numbers on the GC peaks correspond with compound numbers in Table 3. The *x*-axis is the retention time in minutes and the *y*-axis is the abundance.

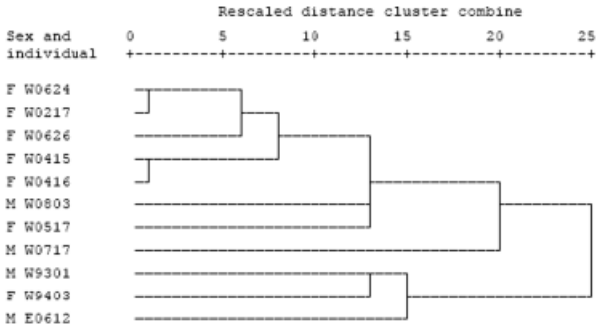

Figure 4 Dendrogram of hierarchical cluster analysis by using squared Euclidean distance for male and female brown bear *Ursus arctos* anal gland secretion. Labels indicate the bears' sex and individual number, and 'M' indicates male and 'F' indicates female.

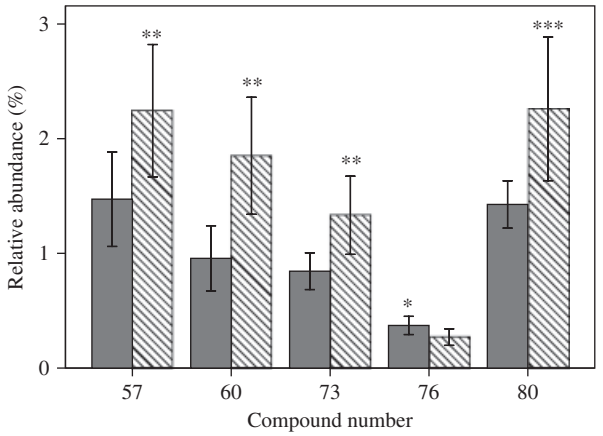

Figure 5 Sex-differences in relative abundance (mean ± SD) of compounds found in the anal gland secretion of male (filled) ($n=4$) and female (hatched) ($n=7$), brown bears *Ursus arctos*. $^{*}P=0.058$ (marginally significant), $^{**}P<0.05$, $^{***}P<0.01$. The numbers on the *x*-axis correspond with compound numbers in Table 3.

sex was not digitally coded in giant pandas. However, our result would have been different if we had used the same definition Zhang *et al.* (2003) used for the Siberian weasel. They concluded that (*Z*)-2-ethyl-3-methylthietane was a sex-specific compound because it was found in seven of 11 females and no males. According to this criterion, we would have defined compound no. 90 (unknown) as sex specific.

The hierarchical cluster analysis failed to show a clear grouping of sex in AGS. However, other information might be found in the digital composition. A similar cluster analysis of AGS from giant pandas revealed a clear grouping between adults and subadults rather than sex (Yuan *et al.*, 2004; Liu *et al.*, 2006). Differences between age groups in brown bears should be investigated in future studies.

Of the compounds detected in the AGS, 68% had a MW above 300. Compounds with MW > 300, the upper limit for airborne pheromones (Wilson, 1963; Bradbury & Vehrencamp, 1998), are well-suited for marking in large home

Table 3 Tentatively identified compounds in anal gland secretion of brown bear *Ursus arctos*

GC peak number	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	Number of individuals		Relative abundance[b]	
				Male ($n=4$)	Female ($n=7$)	Male	Female
1	11.243	Piperidinone	–		3		0.174
2	21.686	Tetradecene (hydrocarbon)	196		1		0.105
3	23.511	Pentadecene (hydrocarbon)	210		1		0.565
4	25.249	Hexadecene (hydrocarbon)	224		1		0.443
5	26.650	Hexadecanoic acid (palmitic acid)	256	2	1	0.606	0.076
6	26.960	Unidentified nitrogen compound	299		1		0.115
7	27.689	*n*-phenyl benzensulfonamide	233	1		0.267	
8	27.702	Unknown	–	2	2	0.485	0.112
9	28.191	Unknown	–	2	1	1.721	0.104
10	28.627	10,13-octadecadienoic acid, methyl ester	294	1		0.056	
11	28.733	Hydrocarbon C21	296	1		0.042	
12	28.818	Unknown	236		1		0.078
13	29.408	Octadecenoic acid (oleic acid)	282	2	1	0.763	0.193
14	29.749	Octadecanoic acid (stearic acid)	284	2	1	0.915	0.094
15	29.809	Hydrocarbon	–	1	3	0.183	0.104
16	30.219	Hydrocarbon C22	310	1		0.066	
17	30.376	Hydrocarbon	–	1		0.074	
18	30.699	Unknown	–	2	2	0.175	0.089
19	31.156	Unknown	226	1		0.483	
20	31.247	Unknown	–	2	1	1.620	0.206
21	31.286	Unknown	236	2	6	0.139	0.084
22	31.411	Unknown	–		1		0.102
23	31.642	Unsaturated wax ester C24	366	1		0.121	
24	31.796	Unknown	–	1		0.055	
25	32.540	Unknown	–	1	4	0.147	0.113
26	32.551	Unknown	–	1		0.252	
27	32.751	Unknown	250	4	7	0.358	0.423
28	32.813	Unknown	–	1	1	0.123	0.086
29	32.900	Unknown	–		2		0.129
30	32.929	Hexanedioic acid, dioctyl ester	370	1		0.274	
31	33.011	Hydrocarbon C24	338	1		0.109	
32	33.220	Unknown	–		2		0.085
33	33.324	Phenol, 2,2′-methylenebis (6-(1,1-dimethylethyl)-4-methyl)	340	1		0.159	
34	33.533	Unknown	–	1		0.284	
35	34.006	Unknown	–	1		0.045	
36	34.152	Unknown	264	3	7	0.628	0.395
37	34.301	Unknown	294	2	3	0.097	0.073
38	35.300	Unknown	324	2		0.103	
39	35.535	Unknown	462	1	3	0.027	0.081
40	36.750	Unknown	292	2		0.083	
41	37.295	Unknown	320	3	3	0.108	0.110
42	37.644	A steroid	368	3	2	0.125	0.121
43	38.362	Squalene	410	1		0.112	
44	38.531	Unknown	334	4	7	0.237	0.299
45	38.771	A steroid	368	3	2	0.188	0.215
46	38.866	A steroid	366	1		0.061	
47	38.911	A steroid	366		1		0.076
48	39.144	A steroid	368	4	7	0.864	0.609
49	39.461	Hydrocarbon	364	3	4	0.231	0.092
50	39.527	Hydrocarbon	364	1		0.142	
51	39.749	A steroid	366	4	7	2.539	3.210
52	40.158	A steroid	–	2	2	0.109	0.094
53	40.555	Unknown	–	1		0.087	
54	40.655	Hydrocarbon	–		1		0.078

Table 3 Continued.

GC peak number	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	Number of individuals		Relative abundance[b]	
				Male ($n=4$)	Female ($n=7$)	Male	Female
55	40.663	Unknown	–	2	2	0.178	0.165
56	40.837	Unknown	–	1	1	0.081	0.102
57	40.984	A steroid	380	4	7	1.472	2.244
58	41.808	Cholesterol	386	4	7	77.289	80.836
59	42.035	Unknown	394	2	4	3.118	3.512
60	42.177	A steroid	384	4	7	0.956	1.851
61	42.288	A steroid	386	3	6	1.181	0.477
62	42.323	A steroid	430	1		0.410	
63	42.409	Unknown	–	2	6	0.292	0.307
64	42.503	A steroid	414	2	2	0.358	0.201
65	42.519	A steroid	414	1		0.174	
66	42.660	Unknown	–		1		0.054
67	42.660	A steroid	–	1		0.103	
68	42.700	A steroid	382		1		0.156
69	42.735	Unknown	–	2	4	0.104	0.110
70	42.787	Ergost -5-en-ol (3β)	400	4	7	0.343	0.396
71	42.908	A steroid	412	3	2	0.224	0.142
72	43.029	A steroid	–	1		0.211	
73	43.066	A steroid	408	4	7	0.845	1.332
74	43.447	A steroid	428	4	7	1.799	0.564
75	43.610	Unknown	–	1		0.090	
76	43.689	A steroid	414	4	7	0.371	0.269
77	43.813	A steroid	–		1		0.078
78	43.868	A steroid	426	1	3	0.275	0.336
79	43.898	Lanosta-8,24-diene-3-ol (3-β) (lanosterol)	426	3	4	2.989	1.216
80	44.115	A steroid	422	4	7	1.427	2.260
81	44.423	A steroid	404	2		0.332	
82	44.486	Unknown	–		3		0.213
83	44.828	Unknown	430	2	1	0.139	0.154
84	44.831	Unknown	–	1		0.117	
85	45.124	Unknown	436	4	7	0.183	0.308
86	45.200	A steroid	–	2		0.205	
87	45.817	Unknown	476	1		0.105	
88	46.191	A steroid	450	1		0.120	
89	46.206	Unknown	–	1		0.155	
90	46.278	Unknown	450		4		0.155

[a]Mean value of the retention time.
[b]Mean value for *n* possessing this compound.
GC, gas chromatogram.

ranges. This suggests that brown bears might use AGS for long-lasting or delayed olfactory communication, as in the giant panda (Yuan *et al.*, 2004). Interestingly, bears have been observed to frequently sit down in front of trees after rubbing and that, when coming to a tree, they often sniff the ground before rubbing (O. -J. Sørensen, pers. comm.).

Our study revealed that brown bear AGS likely codes for sex, and suggests that other vital information is probably encoded in their secretion, enabling the typically solitary bears to communicate indirectly. This increases our knowledge of the repertoire of communication modalities in ursids.

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