År: 09.06.2008

Chemical communication in yellow-bellied marmot (*Marmota flaviventris*) and brown bear (*Ursus arctos*)

Paper 1

Chemical composition of perioral, orbital and anal gland secretion of yellow-bellied marmot (*Marmota flaviventris*): glandular differences and coding for sex

Bård Andreas Lassen and Kristian Ingdal

Paper 2

Do anal gland secretion and feces of brown bear (*Ursus arctos*) code for sex?

Kristian Ingdal and Bård Andreas Lassen

Master thesis

Telemark University College, Scandinavian Brown Bear Project and Rocky Mountain Biological Laboratory

Avdeling for allmennvitenskaplige fag
Hallvard Eikas plass, 3800 Bø

Tittel:	Chemical communication in yellow-bellied marmot (*Marmota flaviventris*) and brown bear (*Ursus arctos*).
Nøkkelord:	Yellow-bellied marmot, *Marmota flaviventris*, brown bear, *Ursus arctos,* glandular secretion, glandular differences, sex differences, gas chromatography-mass spectrometry, digital and analog coding.
Forfattere/	Bård Andreas Lassen, Kristian Ingdal
Studentnr.:	002519, 031744
Fagkode:	4317
Oppgavetype:	Mastergradsoppgave
Studiepoeng:	2x60
Studium:	Mastergrad i Natur-, helse- og miljøvern.
Konfidensiell:	Nei

Avdeling for allmennvitenskaplige fag
Hallvard Eikas plass, 3800 Bø

Preface

This study was initialized when our teaching supervisor Frank Rosell, a specialist within chemical communication, established collaboration with Jon Swenson and Andreas Zedrosser in the Scandinavian Brown Bear Project. They wanted to initiate an innovative and exiting study on chemical communication in brown bears, an unknown topic for this species. At about the same time, Frank also established contact with the experienced scientist Dan Blumstein from the USA. One of his special fields was yellow-bellied marmots in the Rocky Mountains, and he also wanted help with the same topic.

Frank needed assistance for these projects, and a Master thesis was suddenly a reality for us. We conducted our field periods in Sweden and in the Rocky Mountains, USA. During this work we came in contact with a lot of interesting people, and we want to thank the whole crew in both the bear project and the marmot projects for a lot of fun and good memories during the field work. The trip to the USA became even more interesting when Øyvind, Bjørnar and Frank came along.

After the field work, we spent almost 6 months in the lab analyzing the samples that we had collected, and we are glad that Bjørn Steen did not give us up. Further thanks to the cake monsters at the Master lab for making the writing process more inspiring.

Kristian Ingdal Bård Andreas Lassen

Telemark University College

Bø i Telemark

9 June 2008

Paper 1

Chemical composition of perioral, orbital and anal gland secretion of yellow-bellied marmot (*Marmota flaviventris*): glandular differences and coding for sex

Bård Andreas Lassen and Kristian Ingdal

To be submitted to Journal of Chemical Ecology

CHEMICAL COMPOSITION OF PERIORAL, ORBITAL AND ANAL GLAND SECRETION OF YELLOW-BELLIED MARMOT (*MARMOTA FLAVIVENTRIS*): GLANDULAR DIFFERENCES AND CODING FOR SEX

BÅRD ANDREAS LASSEN[1, *] and KRISTIAN INGDAL[1]

[1]*Faculty of Arts and Sciences*

Department of Environmental and Health Studies

Telemark University College

N-3800 Bø i Telemark, Norway

Abstract—Mammals use scent for communication, and olfactory information about sex is often released through specialized scent glands. Yellow-bellied marmots (*Marmota flaviventris)* posses three different scent glands, but little is known about the chemical information in their gland secretion. With a combination of ethanol extraction and gas chromatography and mass spectrometry, we investigated the perioral (PGS), orbital (OGS) and anal gland secretion (AGS) from 9 females and 14 males. We found glandular differences in gas chromatograms of PGS, OGS and AGS, and detected 15, 22 and 21 compounds in PGS, OGS and AGS respectively. AGS contained significantly more compounds than OGS, and marginally significant more than PGS. OGS and PGS did not differ in number of compounds. These results supported the hypothesis that PGS, OGS and AGS have different chemical functions. We did not find any sex differences in gas chromatograms, in number of compounds or in digital (presence/absence of compounds) and analog coding (relative

abundance of shared compounds) of PGS, OGS and AGS. Theses results do not support the hypothesis that PGS, OGS and AGS code for sex. However, we found one marginal significant AGS compound that might code for sex through analog coding.

Key Words─Yellow-bellied marmot, *Marmota flaviventris*, glandular secretion, glandular differences, sex differences, gas chromatography-mass spectrometry, digital and analog coding.

* To whom correspondence should be addressed. E-mail: Baardlassen@yahoo.no

INTRODUCTION

Chemical communication plays an important role in mammalian social and reproductive behavior and many species use olfactory signals to send out information about individual identity, social status, group membership, territory boundaries, sex, age, kin and reproduction state (Wyatt, 2003; Müller-Schwarze, 2006). These signals are commonly released through scent organs, and their functions have been well described for several species of rodents (see Johnston, 2003). In rodents like Eurasian beaver (*Castor fiber*) (Rosell and Sundsdal, 2001) and house mouse (*Mus musculus*) (Zhang et al., 2007) studies of gland secretion revealed e.g. sex differences in chemical composition.

The sources of odors often appear to be secretions from specialized glands (Brown and Macdonald, 1985; Müller-Schwarze, 2006) and for all the studied species of genus Marmota it has been reported two sudoriferous facial glands (Blumstein and Henderson, 1996; Armitage, 2003), a perioral gland (Ouellet and Ferron, 1988) and an orbital gland (Rausch and Bridgens, 1989). The perioral gland is situated bilaterally in the lower lip at the oral angles, while the orbital gland is present bilaterally in the skin of the cheeks between the ear and the eye. Both

glands secrete a yellowish aromatic fluid (Rausch and Bridgens, 1989) and are used for scent marking by rubbing the cheeks and oral angles against rocks, roots and other objects (Armitage, 2003). Marmots also possess anal glands present as three papillae that may be protruded from the anus (Rausch and Bridgens, 1989). The anal glands have a fatty secretion with a strong odor, but their function is unknown.

Most scent related studies in Marmota have focused on rubbing behavior by the two facial glands (e.g. Hébert and Prescott, 1983; Ouellet and Ferron, 1988; Taulman, 1990; Meier, 1991; Bel et al., 1995; Blumstein and Henderson, 1996; Brady and Armitage, 1999). The odor left by this activity seems to have multiple functions like territorial marking, dominance, burrow occupancy, individual identity, familiarity with the home range, and a possible self-assurance role (Armitage, 2003).

Bel et al. (1999) reported that sex of the odor donor did not influence the response of males or females when they investigated orbital gland secretion in alpine marmots *(Marmota marmota*). Similar findings were reported by Meier (1991) who tested the response of adult woodchucks (*M. monax*) to perioral gland secretion. Blumstein and Henderson (1996) reported on the other hand that adult males in golden marmots (*M. caudate aurea*) responded more vigorously to the orbital gland secretion of non-group females than of non-group males. This suggests that males may be able to distinguish the sex of potential signalers from their orbital gland secretion. However, no studies have investigated sex differences in response of yellow-bellied marmots (*M. flaviventris*) to secretion from conspecific males and females. Despite several scent related behavioral studies, there has only been carried out one chemical study of gland secretion in the Marmota. Bel et al. (1999) investigated the chemical composition of orbital gland secretion in alpine marmots by gas chromatography-mass spectrometry, and reported that the differences appeared to be unrelated to gender.

It seems to be various contradictory findings in Marmota when it comes to sex differences in gland secretion, and this should therefore be investigated further. Especially chemical analysis of compound composition is useful to reveal sex related information in scent. So far, no studies have investigated perioral, orbital and anal gland secretion of the same Marmota species by chemical analysis.

In this study we investigated the chemical composition of gland secretions in male and female yellow-bellied marmots. We hypothesized that perioral (PGS), orbital (OGS) and anal gland secretion (AGS) have different chemical functions, and predicted that PGS, OGS and AGS differ in gas chromatograms (GC) and in number of compounds. We also hypothesized that PGS, OGS and AGS code for sex. We predicted that PGS, OGS and AGS have sex differences in GC, in number of compounds and in digital (presence/absence of compounds) and analog (relative abundance of shared compounds) coding.

METHODS AND MATERIALS

Study Area and Animals. We conducted the study in Upper East River Valley, Gunnison County, CO, USA from June 19 - 28, 2007. The yellow-bellied marmots were trapped at eight different colonies near Rocky Mountain Biological Laboratory in Gothic (38° 57´ 29´´ N, 106° 59´ 06´´ E) (Table 1). The habitat varied within and between sites from rolling grassy meadows to steeper talus, and the marmots had a patchy distribution in sub-alpine meadows and forest openings (Blumstein et al., 2006). The yellow-bellied marmot is a hibernating, diurnal rodent that lives in organized kin-groups of closely related females and an attached male. Each individual group is territorial and lives in burrows (Armitage, 2003). The species is widely distributed in the western North America (Frase and Hoffmann, 1980; Armitage, 2003).

We live-trapped marmots (female $N = 9$, male $N = 14$) by using Tomahawk live-traps

(Tomahawk, WI 54487, USA) set close to borrow entrances. Once trapped, each animal was transferred to a canvas handling bag in which it was sexed and aged before taking scent samples (Table 1). The sex of the animal was determined by measuring the distance between anus and genitals, while age class (adult/yearling) was set from body weight (Armitage et al., 1976).

Scent Sample Collection. PGS (N = 11) and OGS (N = 15) were collected by rubbing the glands with sterile, wooden Q-tips. This was done through the front opening of the handling bag. The marmot was kept steady during collection by another person who gently pressed its head and neck against the ground. To collect AGS (N = 22), we held the marmot on its back, opened the rear opening of the handling bag and exposed the anus area. While one person protruded the anal glands, another person squeezed and rubbed the openings of the three papillae with a Q-tip. The anal glands were pre-cleaned with alcohol to avoid feces from contaminating the samples. We also collected control samples rubbed in the fur of neck. After collection, each Q-tip was placed in a labeled and dated 40 ml glass vial with teflon lined cap (Lab Safety Supply®, WI 53547-1368, USA), and immediately placed on ice. All samples were frozen at -20°C within 2 hr of collection, and kept frozen until analysis. We used latex gloves while collecting the samples to avoid contamination of human odor. The samples were air-shipped to Norway, placed on dry ice in a cooling box. We checked the box after arrival, and all samples were still frozen.

Chemical Sample Preparation. We removed the cotton with scent from the Q-tip with a sterile needle, and put it in a small glass test tube. We added 1 ml 99% ethanol and vortexed for 15 sec. Different solvents were tried in a pre-study, but we used ethanol, which have been reported to extract more OGS compounds than pentane and dichloromethane (Bel et al., 1999). The compounds were extracted for 2 hr, before 2/3 of the solution was pipetted into a 2 ml GC-vial. The test tube was covered with aluminum foil during the extraction, to avoid

chemical contamination and loss of volatile compounds. All samples were prepared at the same time and analyzed within 24 hr. Prior to use, all lab equipment were washed properly, cleaned in acetone and baked for 12 hr at 200°C. This was done to avoid contamination of the samples.

Chemical Analysis. We used an auto-injection system (Agilent 7683 Series Injector) to inject 1 µl of the sample extraction into a Hewlett-Packard (HP) 6890 Series II gas chromatograph. The gas chromatograph was equipped with a single taper HP liner (4 mm inner diameter) with glass wool and a non-polar HP-5 MS 5 % phenyl-methyl-siloxane capillary column (30 m x 0.25 mm x 0.25 µm film thickness). A HP 5973 Series mass selective detector with a split/splitless inlet was connected and used in the splitless mode. Front inlet temperature was set to 270°C. We used helium as the carrier gas at constant flow of 1.0 ml/min, and purge flow to split vent was 49.8 ml/min@1.00 min. The initial oven temperature was set to 40°C, and then increased 8°C/min to 320°C, which was maintained for 5 min. Total run time was 44.0 min. We used a solvent delay of 5 min for every run, to avoid that the solvent damaged the detector. We re-analyzed 24 hr old extractions from two males and two females, to check that no compounds disappeared during this period. No loss of compounds was observed. We ran blank samples with ethanol before, in the middle and after the scent samples to control for changes in retention time. No major changes was observed.

We tentatively identified each compound from retention time and mass spectrum of its GC-peak. Most of the compounds were determined by comparing the structure with known compounds in Wiley 275 Library, which contain about 70,000 known compounds. This was done by a computer-aided compound search. Structures of unidentified compounds were added in a new library, which we included in the computer search. The new compounds could then be recognized in different samples by comparing structures and retention times. All mass spectra from the samples and the libraries were visually compared to make sure that the

computer mass spectrometry suggestions were reasonable. Since the main focus of our study was to check if gland secretion code for information about sex, positive identification with known standards was not attempted for any of the compounds. Area of each peak was found by using the computer aided integration with threshold set to 16.0. This value was chosen to avoid integration of small peaks that originated from the background noise.

We compared all samples of PGS and OGS with control samples to make sure that the marmot fur did not contribute with any chemical compounds in the data analyses. We did not do this for the AGS samples, since the anal glands are not in contact with the marmot fur. We removed all the peaks that originated from the solvent, column and fur, before further data analyses.

Statistics. In order to test whether PGS, OGS and AGS differed in GCs, we used partial least squares (PLS1) regression formerly used successfully by Rosell and Steifetten (2004). PLS1 is a multivariate calibration method that uses the information in Y-matrix actively to find the Y-relevant structure in X-matrix (Esbensen, 2002) (for details, see Rosell and Steifetten, 2004). As a basis for comparison, the abundance of total ion detection was measured for every time unit (165 time units/min) on the retention scale of the GC. The measured values formed a GC-matrix of X-variables (5977 time measurements) and Y-variables (gland type of 48 scent samples). Use of PLS1 was appropriate for analyzing the GC-matrix, because the X-variables were strongly inter-correlated and the number of variables was greater than the number of samples (Wold et al., 1983). All values were scaled by mean normalization to minimize the effect of considerable variation in abundance between the samples. Before we ran the model, we removed the first 481 time measurements because they contained information that originated from the solvent. The method extracts a small number of PLS1 components (PCs) which represent the relevant latent dimensions of the model. We used the values of validated R-square and root mean square error of prediction

(RMSEP) to evaluate the results. Validated R-square tells about the predictive ability of the model, and the closer to 1, the better. RMSEP is a measurement of the average difference between predicted and measured response values, at the prediction or validation stage. A value closer to 0, the better. The statistical software used was The Unscrambler 9.7 (CAMO Software AS).

Gland secretions can according to Sun and Müller-Schwarze (1998a, b) contain information in digital and analog form. Since marmots probably not are able to control for the exact abundance of scent secreted, the absolute abundance of each compound is unlikely to be used to code for sex. Therefore we investigated both absence/presence and relative abundance for the compounds.

We digitally encoded the detected compounds by 0 (absent) or 1 (present) for PGS, OGS and AGS from all individuals. The data formed a digital matrix of X-variables (detected compounds) and Y-variables (individuals) for each gland. From this data we could find the number of detected compounds within individuals, gender and glands. A compound was defined to be sex specific if found in all males or females (Andersen and Vulpius, 1999). We used the nonparametric Kruskal-Wallis test with Bonferroni post hoc test to check for glandular differences in number of detected compounds in the individuals. To check for similarities within sex in digital coding of PGS, OGS and AGS, we performed a hierarchical cluster analysis with squared Euclidean distance (Yuan et al., 2004) on the digital matrixes. We used cluster analysis because the digital matrix contained more variables (number of compounds) than observations (number of individuals), and thus, canonical discriminant analysis was inappropriate (Johnson and Wichern, 1992).

To check for analog coding, we calculated the relative abundance for each peak as the percentage of the total area of the GC-peaks. Sex differences in relative abundance were investigated for compounds that were shared by at least four males and four females. This was

done by using a two-tailed Mann-Whitney *U* test, which requires minimum four observations of each sex (Zar, 1998). Due to low sample size, we were only able to investigate analog sex differences for six compounds in AGS and one compound in PGS. We used only nonparametric statistics since the data did not fit assumptions of distributions and homogeneity of variance for parametric analysis (Sokal and Rohlf, 1995). We set significant level to $P < 0.05$, and defined a *P*-value between 0.05 - 0.1 as marginal significant (see Zhang et al., 2008). Statistical analyses were conducted by using SPSS for Windows (version 15.0; SPSS Inc.) and Analyse-it® Standard Edition (Analyse-it Software, Ltd).

RESULTS

Glandular Differences. Of a total of 43 different tentatively identified compounds, we found 15 compounds in PGS ($\bar{X} \pm SD = 3.90 \pm 2.43$), 22 compounds in OGS ($\bar{X} \pm SD = 3.00 \pm 3.52$) and 21 compounds in AGS ($\bar{X} \pm SD = 5.72 \pm 2.25$) (Table 2). It was a significant difference between number of compounds in PGS, OGS and AGS (Kruskal-Wallis test, $H = 15.59$, $df = 2$, $P < 0.001$, $N_{PGS} = 11$, $N_{OGS} = 15$, $N_{AGS} = 22$). Bonferroni post-hoc test showed that AGS contained more compounds than OGS ($P < 0.001$), and marginally significant more than PGS ($P = 0.074$). OGS and PGS did not differ significantly in number of compounds ($P = 0.24$). When investigating males and females separately, we found significant differences in number of compounds between OGS, PGS and AGS in both females ($H = 9.21$, $df = 2$, $P = 0.01$, $N_{PGS} = 4$, $N_{OGS} = 6$, $N_{AGS} = 9$) and males ($H = 6.81$, $df = 2$, $P = 0.03$, $N_{PGS} = 7$, $N_{OGS} = 9$, $N_{AGS} = 13$). Bonferroni post-hoc test revealed that AGS contained more compounds than OGS (males $P = 0.02$, females $P = 0.003$), male AGS did not contain more compounds than male PGS (males $P = 0.67$), and female AGS contained marginally significant more compounds than female PGS ($P = 0.08$). OGS and PGS did not differ in number of compounds in neither males ($P = 0.59$) nor females ($P = 1.0$).

A comparison of GCs by PLS1 showed differences in composition between PGS, OGS and AGS. The OGS and PGS samples were clustered into two obvious groups, while the AGS samples were more scattered (Figure 1). Despite some overlap, the groups showed a clear tendency of separation. Of the total variation within all GC, PC 1 explained 75% of the X-variance and 42% of the Y-variance, while PC 2 explained 13% of the X-variance and 42% of the Y-variance. Validated R-square = 0.84, and RMSEP = 0.33.

GC Comparison of Sex Differences. A visual examination of male and female GCs showed no consistent qualitative sex differences in neither of the glands, i.e. it seemed to be a great variation in the presence and absence of peaks between the individuals. Figure 2a-f show typical GCs of PGS, OGS and AGS from adult males and females.

Digital Coding. In PGS we found six compounds (no. 5, 6, 10, 21, 22 and 28) only in males, but no compounds were found in all of them. Compound no. 5 was present in five of seven males. Three compounds (no. 12, 13 and 38) were only found in females, but none of these were shared by more than one individual. Six compounds (no. 8, 9, 31, 33, 41 and 43) were shared by both sexes (Table 2). We found no sex differences in number of compounds in PGS (Mann-Whitney U test, $U = 10.5$, $N_{\text{males}} = 7$, $N_{\text{females}} = 4$, $P = 0.50$).

In OGS we found 17 of 22 compounds (no. 1, 2, 3, 4, 5, 7, 10, 18, 19, 21, 22, 24, 26, 28, 33, 36 and 42) in males only, but none of these were shared by more than one individual. One compound (no. 34) was only found in a female and four compounds (no. 8, 9, 31 and 37) were shared by males and females (Table 2). Compound no. 31 was the only compound that was found in at least four males and four females. We found no sex differences in number of compounds in OGS ($U = 26.5$, $N_{\text{males}} = 9$, $N_{\text{females}} = 6$, $P = 0.95$).

In AGS we found eight of 21 compounds (no. 14, 16, 17, 23, 29, 30, 35 and 39) in males only. Compound no. 35 was found in four males, while compound no. 16, 23 and 26 were found in two males. One compound (no. 25) was only found in females, but only in one

animal. We found 12 compounds (no. 8, 9, 11, 12, 13, 15, 20, 27, 31, 32, 38 and 40) that were shared by males and females, and six of these were present in at least four males and four females (compound no. 8, 9, 13, 31, 38 and 40) (Table 2). Compound no. 31 and 40 were found in all the animals. We found no sex differences in number of compounds in AGS (U = 13.0 N_{males} = 13, $N_{females}$ = 9, P = 0.81). The hierarchical cluster analyses revealed no obvious separation between males and females in neither of the gland secretions (Figure 3a-c).

Analog Coding. We found that females had a marginal significant higher relative abundance of compound no. 9 (hexadecanoic acid, ethyl ester) (U = 55.0, N_{males} = 8, $N_{females}$ = 7, P = 0.083) in AGS. We found no sex differences in relative abundance of the other five shared compounds in AGS (Mann-Whitney U test, all P > 0.14). The shared compound in OGS (no. 31) did not either differ in relative abundance between males and females (U = 14.0, N_{males} = 5, $N_{females}$ = 6, P = 0.56).

DISCUSSION

This study is the first to investigate chemical compounds and composition of gland secretion in yellow-bellied marmots. Our results supported the hypothesis that PGS, OGS and AGS have different chemical functions. PGS, OGS and AGS differed in GC and in number of compounds, as we predicted. The study did not support the hypothesis that PGS, OGS and AGS code for sex, and neither of the predictions were supported. However, we found one marginal significant AGS compound that might code for sex through analog coding.

Glandular differences like we found in this study were also reported from ringtailed lemur (*Lemur catta*) where three different glands were compared (Scordato et al., 2007). They concluded that the lemur glands sent out different kind of information, and we suggest the same for PGS, OGS and AGS in yellow-bellied marmots. The PLS1 showed that AGS varied much more in composition than PGS and OGS that had less inter-glandular variation. This

might imply that AGS has a more individual specific composition, or simply just contain a broader range of compounds.

The fact that AGS also seem to contain more chemical compounds than PGS and OGS can indicate that anal glands are used for some kind of scent marking, unlike what earlier has been suggested by Armitage (1974) and Rausch and Bridgens (1989). They stated that anal glands were not used for scent marking, but in agnostic displays and under conditions of stress and anger. Scent marking by AGS is on the other hand supported by Bopp (1954) who found deposited AGS from alpine marmots near logs in different study colonies. Also the woodchucks use their anal glands, and Haslett (1973) suggested that the released scent might serve as an alarm signal that inhibits the activity of conspecifics. To better understand the function of anal glands, AGS should be investigated further by behavioral and chemical studies of a larger number of animals.

In contrast to chemical studies of for example house mouse (Zhang et al., 2007) and steppe polecat (*Mustela eversmanni*) (Zhang et al., 2003), we did not find any significant sex differences in analog coding of shared compounds in marmot odor. However, compound no. 9 had a marginal significant higher relative abundance in female AGS, and would probably be significant with a larger sample size. This compound (hexadecanoic acid, ethyl ester) might therefore be important for sex discrimination in marmots. Similar studies of scents from e.g. giant panda (*Ailuropoda melanoleuca*) (Zhang et al., 2008) and white-tailed deer (*Odocoileus virginianus*) (Gassett et al., 1996) found differences in analog coding by using 0.1 as significant level. We discussed this option, but even though this would have given significant differences in analog coding of AGS, we chose to use the most common level (0.05) in this kind of chemical studies. However, our results imply that AGS might code for sex through analog coding.

In contrast to Rosell and Sundsdal (2001) who found sex related GC patterns in AGS from Eurasian beaver, we could not recognize any visual differences in GCs between male and female PGS, OGS and AGS. A visual examination is nevertheless a limited method for comparing GCs. A multivariate calibration method like PLS1 would probably be more appropriate for comparing GC from each gland (Esbensen, 2002). Rosell and Steifetten (2004) found clear sex differences in anal gland secretion of Eurasian beaver and North American beaver (*C. canadensis*) by using PLS1. Unfortunately, our data did not fit this regression model due to low residual validation variance.

We found relatively few compounds in the OGS compared to what Bel et. al (1999) found in alpine marmots. They identified 30 compounds that were found in at least 50% of 24 OGS samples from alpine marmots. This is in strong contrast to the one single OGS compound that we identified in more than half of our animals. However, there might be great differences in number of detected compounds in gland secretion of different species. Even within the three species of the Hyaenidae family did Buglass et al. (1990) find differences in number of detected compounds. They found nine compounds in brown hyena (*Hyena brunnea*), eight compounds in spotted hyena (*Crocuta crocuta*), but only two compounds were found in striped hyena (*H. hyena*). These results are in accordance with the differences within the Marmota species.

The digital encoding revealed several compounds that were only found in one gender. However, most of them were only found in one or two individuals, and none were present in all the animals of a particular sex. Therefore, it is difficult to say whether any of these compounds really code for sex. We would expect that compounds that code for maleness would be present in all the males, but this was not found in our study. It is still possible that different age classes have unequal sex specific compounds. Yearling yellow-bellied marmots have been reported to scent mark with the perioral glands, but however, this is most common

among dominant males and females (Armitage, 1976). The adults might therefore have a more developed scent containing more information than scent from yearlings. This is supported by Rausch and Bridgens (1989) who reported that both orbital gland and perioral glands did not attain full development in marmots less then tree years of age. We did not separate between yearlings and adults in our analysis, but this should be done with a larger sample size.

The compounds detected in only one or two individuals are probably less likely to be an important part of chemical communication than those that occur regularly. This means that 23 of 43 compounds do probably not play an important role. Compound no. 31 (cholest-5-en-3-ol) and no. 40 (a steroid) were in contrast found in AGS from all the investigated animals, and we also found compound no. 35 (a steroid) in AGS from four of 13 males and compound no. 5 (ethyl laurate) in PGS from five of seven males. Even if these compounds do not code for sex, they might have an important function within the species. Our findings imply that information about sex is not digitally coded in any of the three gland secretions, and this is supported by the lack of sex related separation between the individuals in the hierarchical cluster analyses. Contrary to what we expected, we did not find any of the compounds that Bel et al. (1999) found by chemical analysis of OGS from alpine marmots.

Behavioral studies of OGS in alpine marmots (Bel et al., 1999) and PGS in woodchucks (Meier, 1991) support our findings by suggesting that secretions from these glands do not contain information about sex. Most likely is scent marking by PGS and OGS in yellow-bellied marmots used for other purposes. Brady and Armitage (1999) reported that marmot scent marking by PGS is a multipurpose activity that provides cues for young of the year to learn safe areas in the home range, imparts familiarity with the burrow area, communicates burrow occupancy, and functions in territorial defense. The orbital gland is used for scent marking through cheek-rubbing by territorial males who defend their territory

(Armitage, 1974, 1976). This can imply that PGS and OGS from yellow-bellied marmots code for information about family, group or individual rather than sex.

Even though no information about sex was found in PGS and OGS, marmots might send out sex related cues in other ways. The composition of mammalian glandular secretion can vary a lot within a species, and might code for information about e.g. sex, age, individuality, kin or group through a complex mixture of chemical compounds (Albone, 1984; Brown and Macdonald, 1985). This means that biological information about the scent donor might be coded through a complex mix of compounds of varying abundance (Albone, 1984). This will not be detected through analog and digital coding, but might be revealed by field bioassays.

It is reasonable to question if we used the right solvent for extracting compounds. We detected far less compounds in OGS than Bel et al. (1999), even if we used the solvent that they found was best for extracting OGS compounds from alpine marmots. By conducting a more thorough pre-study of different solvents, more compounds might be detected. Bel et al. (1999) also used scents that were taken from anaesthetized animals. This might give a chance of getting better samples with more secretion on the Q-tips. Another opportunity is to collect scent from glands of recently died animals. We suspect that the collected amount of secretion have been too small in this study. However, scent collection of PGS and OGS from live-trapped marmots is a procedure that is formerly used for behavior studies with good results (Blumstein and Henderson, 1996; Brady and Armitage, 1999).

This study has shown that yellow-bellied marmots have differences in chemical composition of the gland secretion. This suggests that that PGS, OGS and AGS might be used for different purposes. We have also found that AGS might code for sex, but this should be investigated further be behavioral and chemical studies.

Acknowledgements— We thank Bjørnar Hovde, Øyvind Steifetten and Dan Blumstein with his crew at Rocky Mountain Biological Laboratory for excellent assistance in the field. We also thank Bjørn Steen for his assistance with GC-MS analyses, and our supervisor Frank Rosell for good methodical advice, field assistance and helpful comments on earlier drafts of the manuscript. The study was financially supported by Telemark University College.

REFERENCES

ALBONE, E. S. 1984. Mammalian Semiochemistry. John Wiley, New York.

ANDERSEN, K. F. and VULPIUS, T. 1999. Urinary volatile constituents of the lion, *Panthera leo*. *Chem. Senses* 24:179-189.

ARMITAGE, K. B. 1974. Male behaviour and territoriality in the yellow-bellied marmot. *J. Zool. Lond.* 172:233-265.

ARMITAGE, K. B. 1976. Scent marking by yellow-bellied marmots. *J. Mammal.* 57:583-584.

ARMITAGE, K. B. 2003. Marmots, pp. 188-210, in G. A. Feldhamer, B. C. Thompson, and J. A. Chapman (eds.). Wild Mammals of North America. Biology, Management and Conservation. The Johns Hopkins University Press, Baltimore, Maryland.

ARMITAGE, K. B., DOWNHOWER, J. F., and SVENDSEN, G. E. 1976. Seasonal changes in weights of marmots. *Am. Midl. Nat.* 96:36-51.

BEL, M. C., COULON, J., SRENG, L., ALLAINÉ, D., BAGNÉRES, A. G., and CLÉMENT, J. L. 1999. Social signals involved in scent-marking behavior by cheek-rubbing in alpine marmots (*Marmota marmota*). *J. Chem. Ecol.* 25:2267-2283.

BEL, M. C., PORTERET, C., and COULON, J. 1995. Scent deposition by cheek rubbing in the alpine marmot (*Marmota marmota*) in the French Alps. *Can. J. Zool.* 73:2065-2071.

BLUMSTEIN, D. T. and HENDERSON, S. J. 1996. Cheek-rubbing in golden marmots (*Marmota caudata aurea*). *J. Zool. Lond.* 238:113-123.

BLUMSTEIN, D. T., OZGUL, A., YOVOVICH, V., VAN VUREN, D. H., and ARMITAGE K. B. 2006. Effect of predation risk on the presence and persistence of yellow-bellied marmot (*Marmota flaviventris*) colonies. *J. Zool. Lond.* 270:132-138.

BOPP, P. 1954. Zur Topographie der Murmeltierterritorien. *Rev. Suisse Sool.* 63:255-261.

BRADY, K. M. and ARMITAGE, K. B. 1999. Scent-marking in the yellow-bellied marmot (*Marmota flaviventris*). *Ethol. Ecol. Evol.* 11:35-47.

BROWN R. E. and MACDONALD D. W. 1985. Social Odors in Mammals. Oxford University Press, Oxford.

BUGLASS, A. J., DARLING, F. M. C., and WATERHOUSE, J. S. 1990. Analysis of the anal sac secretion of the Hyaenidae, pp. 65-69, in D. W. Macdonald (ed.). Chemical Signals in Vertebrates 5. Oxford University Press, Oxford.

ESBENSEN, K. H. 2002. Multivariate Data Analysis - in Practice: An Introduction to Multivariate Data Analysis and Experimental Design. 5th ed. Camo, Oslo.

FRASE, B. A. and HOFFMANN, R. S. 1980. *Marmota flaviventris*. *Mamm. Species* 135:1-8.

GASSETT, J. W., WIESLER, D. P., BAKER, A. G., OSBORN, D. A., MILLER, K. V., MARCHINTON, R. L., and NOVOTNY, M. 1996. Volatile compounds from interdigital gland of male white-tailed deer (*Odocoileus virginianus*). *J. Chem. Ecol.* 22:1689-1696.

HASLETT, G. W. 1973. The significance of anal scent marking in the eastern woodchuck. *Bull. Ecol. Soc. Amer.* 54:43-44.

HÉBERT, P. and PRESCOTT, J. 1983. Étude du marquage olfactif chez la marmotte commune (*Marmota monax*) en captivité. *Can. J. Zool.* 61:1720-1725.

JOHNSON, R. A. and WICHERN, D. W. 1992. Applied Multivariate Statistical Analysis. Prentice-Hall Inc., Englewoods Cliffs, New Jersey.

JOHNSTON, R. E. 2003. Chemical communication in rodents: From pheromones to individual recognition. *J. Mammal.* 84:1141-1162.

MEIER, P. T. 1991. Response of adult woodchucks (*Marmota monax*) to oral-gland scents. *J. Mammal*. 72:622-624.

MÜLLER-SCHWARZE, D. 2006. Chemical Ecology of Vertebrates. Cambridge University Press, Cambridge.

OUELLET J. P. and FERRON J. 1988. Scent-marking behavior by woodchucks (*Marmota monax*). *J. Mammal.* 69:365-368.

RAUSCH, R. L. and BRIDGENS J. G. 1989. Structure and function of sudoriferous facial glands in nearctic marmots, Marmota spp. (Rodentia: Sciuridae). *Zool. Anz.* 223:265-282.

ROSELL, F. and STEIFETTEN, Ø. 2004. Subspecies discrimination in the Scandinavian beaver (*Castor fiber*): combining behavioral and chemical evidence. *Can. J. Zool*. 82:902-909.

ROSELL, F. and SUNDSDAL, L. J. 2001. Odorant source used in Eurasian beaver territory marking. *J. Chem. Ecol.* 27:2471-2491.

SCORDATO, E. S., DUBAY, G., and DREA, C. M. 2007. Chemical composition of scent marks in the ringtailed lemur (*Lemur catta*): Glandular differences, seasonal variation, and individual signatures. *Chem. Senses* 32:493-504.

SOKAL, R. R. and ROHLF, F. J. 1995. Biometry. 3rd ed. W. H. Freeman and Company. New York.

SUN, L. and MÜLLER-SCHWARZE, D. 1998a. Anal gland secretion codes for family membership in the beaver. *Behav. Ecol. Sociobiol.* 44:199-208.

SUN, L. and MÜLLER-SCHWARZE, D. 1998b. Anal gland secretion codes for relatedness in the beaver, *Castor canadensis*. *Ethology* 104:917-927.

TAULMAN, J. F. 1990. Observations on scent marking in hoary marmots, *Marmota caligata*. *Can. Field. Nat*. 104:479-482.

WOLD, S., MARTENS, H., and WOLD, H. 1983. The multivariate calibration problem in chemistry solved by the PLS method, pp. 286-293, in B. Kågstrøm and A. Ruhe (eds.). Lecture Notes in Mathematics. Vol. 973. Springer-Verlag, Berlin.

WYATT, T. D. 2003. Pheromones and Animal Behaviour, Communication by Smell and Taste. Cambridge University Press, Cambridge.

YUAN, H., LIU, D., SUN, L., WEI, R., ZHANG, G., and SUN, R. 2004. Anogenital gland secretions code for sex and age in the giant panda, *Ailuropoda melanoleuca*. *Can. J. Zool*. 82:1596-1604.

ZAR, J. H. 1998. Biostatical Analysis. 4th ed. Prentice-Hall, New Jersey.

ZHANG, J. X., LIU, D., SUN, L., WEI, R., ZHANG, G., HONGLIN, W., ZHANG, H., and ZHAO, C. 2008. Potential chemosignals in the anogenital gland secretion of giant pandas, *Ailuropoda melanoleuca,* associated with sex and individual identity. *J. Chem. Ecol.* 34:398-407.

ZHANG, J. X., NI, J., REN, X. J., SUN, L., ZHANG, Z. B., and WANG, Z. W. 2003. Possible coding for recognition of sexes, individuals, and species in anal gland volatiles of *Mustela eversmanni* and *M. sibirica*. *Chem. Senses* 28:381-388.

ZHANG, J. X., RAO, X. P., SUN, L., ZHAO, C. H., and QIN, X. W. 2007. Putative chemical signals about sex, individuality and genetic background in the preputial gland and urine of the house mouse (*Mus musculus*). *Chem. Senses* 32:293-303.

TABLE 1. PERIORAL, ORBITAL AND ANAL GLAND SECRETION FROM YELLOW-BELLIED MARMOTS (*N = 23)* OF DIFFERENT SEX, AGE, AGE CLASS AND REPRDUCTIVE STATUS

						Samples collected		
Individual[a]	Sex[b]	Age	Age class[c]	Status	Colony	Perioral	Orbial	Anal
No ear	F	6	A	Reproductive	Beaver Tallus	x	x	x
4682	M	5	A	Reproductive	River	x	x	x
4340	M	4	A	Possibly reproductive	Horse/mound	x	x	x
4121	F	3	A	Reproductive	Horse/mound		x	x
4655	M	3	A	Possibly reproductive	Marmot meadow	x	x	
4657	F	3	A	Reprodutive	River/ bench			x
4691	M	3	A	Possibly reproductive	Bench		x	x
5113	F	3	A	Reproductive	Town			x
5854	F	3	A	Reproductive	Stonefield		x	x
4447	M	2	A	Reproductive	River/bench		x	x
4964	F	2	A	Reproductive	Horse/mound	x	x	x
4187	M	1	Y	Reproductive	Marmot meadow		x	x
4337	M	1	Y	Reproductive	Stonefield	x		x
4517	M	1	Y	Reproductive	Marmot meadow	x	x	x
4754	M	1	Y	Reproductive	River	x		x
4867	M	1	Y	Reproductive	River		x	x
4963	F	1	Y	Reproductive	Marmot meadow	x		x
5059	M	1	Y	Reproductive	Marmot meadow		x	x
5167	M	1	Y	Reproductive	Bench	x		x
5287	M	1	Y	Reproductive	Marmot meadow			x
5735	M	1	Y	Reproductive	Marmot meadow			x
5853	M	1	Y	Reproductive	Bench	x	x	x
6000	F	1	Y	Reproductive	River/bench		x	x

[a] Left ear tag.
[b] M = male, F = female.
[c] Yearling < 2 kg, adult > 2 kg (Armitage et al., 1976).

TABLE 1. TENTATIVELY IDENTFIED COMPOUNDS IN PERIORAL, ORAL AND ANAL GLAND SECRETION AND THEIR FREQENCIES IN MALE AND FEMALE YELLOW-BELLIED MARMOTS, *MARMOTA FLAVIVENTRIS (N = 23)*

GC peak no.	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	Perioral gland		Orbital gland		Anal gland	
				♀(*N*=4)	♂(*N*=7)	♀(*N*=6)	♂(*N*=9)	♀(*N*=9)	♂(*N*=13)
1	13.608	2-phenylethanethiol	138				1		
2	15.650	Benzenepropanethiol	152				1		
3	17.287	Long hydrocarbonchain	198				1		
4	17.397	Ethyl caprate	200				1		
5	20.450	Ethyl laurate	228		5		1		
6	22.828	Unknown	346		1				
7	23.200	Tetradecanoic acid	256				1		
8	24.216	1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester	278	2	3	3	2	4	6
9	25.715	Hexadecanoid acid, ethyl ester	284	3	4	3	4	7	8
10	26.579	Manool	290		2		1		
11	27.202	Unknown	311					1	1
12	27.663	Linoleic acid, ethyl ester	308	1				1	1
13	27.728	9-octadecenoic acid	310	1				6	5
14	27.997	Long hydrocarbonchain	322						1
15	28.008	Octadecanoic acid, ethyl ester	312					3	2
16	28.052	Elaidinic acid, isopropylester	324						2
17	28.528	Unknown	322						1
18	29.878	Unknown	340				1		
19	29.919	Unknown	342				1		
20	30.443	Phenol, 2,2`-methylenebis [6-(1,1-dimethylethyl)-4-methyl	340					1	2
21	31.840	A wax ester, tridecyl undecanoate	368		1		1		
22	33.667	A wax ester, tridecyl pentadecanoate	396		2		1		
23	33.762	A steroid	368						2
24	34.715	Unknown	366				1		
25	34.728	Unknown	366					1	
26	34.893	A steroid	368				1		
27	34.904	A steroid	368					1	4
28	35.355	A wax ester, tetradecyl tetradecanoate	424		2		1		
29	36.294	Unknown	400						2
30	36.484	Unknown	429						1
31	36.730	Cholest-5-en-3-ol	386	1	4	5	6	9	13
32	36.819	Dihydrocholesterol	388					1	2
33	36.944	A wax ester, hexadecyl tetradecanoate	452	1	2		1		
34	37.464	A steroid	382			1			
35	37.630	A steroid	400						4
36	37.766	A steroid	416				1		
37	38.755	Unknown	424			1	3		
38	38.773	A steroid	424	1				4	7
39	38.782	Unknown	424						1
40	39.025	A steroid	410					9	13
41	39.409	Unknown	468	2	1				
42	39.577	Unknown	480				1		
43	39.686	Unknown	468	1	3				
		Total number of compounds		9	12	5	21	13	20

[a] Mean value of the retention times.

Figure legends

Figure 1

Partial least square (PLS1) regression score plots showing the position of each gas chromatograms of yellow-bellied marmots (*Marmota flaviventris*) on the first two components for perioral ● (*N* = 11), orbital □ (*N* = 15) and anal gland secretion ▼ (*N* = 22).

Figure 2

Typical gas chromatograms of perioral (a = male, b = female), orbital (c = male, d = female) and anal (e = male, f = female) gland secretion in adult yellow bellied marmots (*Marmota flaviventris*). The *x* axis is time in minutes, and *y* axis is abundance. Peak marked with numbers in the chromatograms are tentatively identified as: 5, ethyl laurate; 8, 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester; 9, hexadecanoic acid, ethyl ester; 10, manool; 12, linoleic acid, ethyl ester; 13, 9-octadecenoic acid; 24, unknown; 25, unknown; 30, unknown; 31, cholest-5-en-3-ol; 33, a wax ester, hexadecyl tetradecanoate; 34, a steroid; 35, a steroid; 36, a steroid; 37, unknown; 38, a steroid; 39, unknown and 40, a steroid.

Figure 3

Dendrogram of hierarchical cluster analysis with squared Euclidean distance for perioral (a), orbital (b) and anal gland secretion (c) from yellow-bellied marmots (*Marmota flaviventris*). Labels indicate the sex and individual numbers (left ear) of the marmots. “M” indicates male and “F” indicates female.

Figure 1

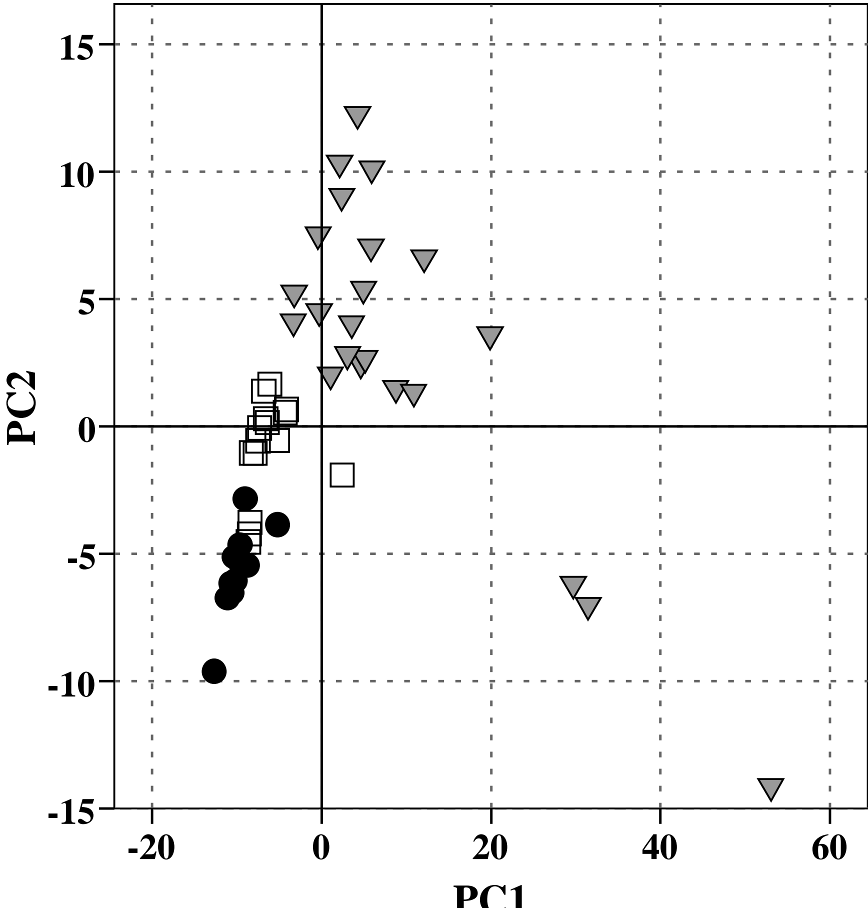
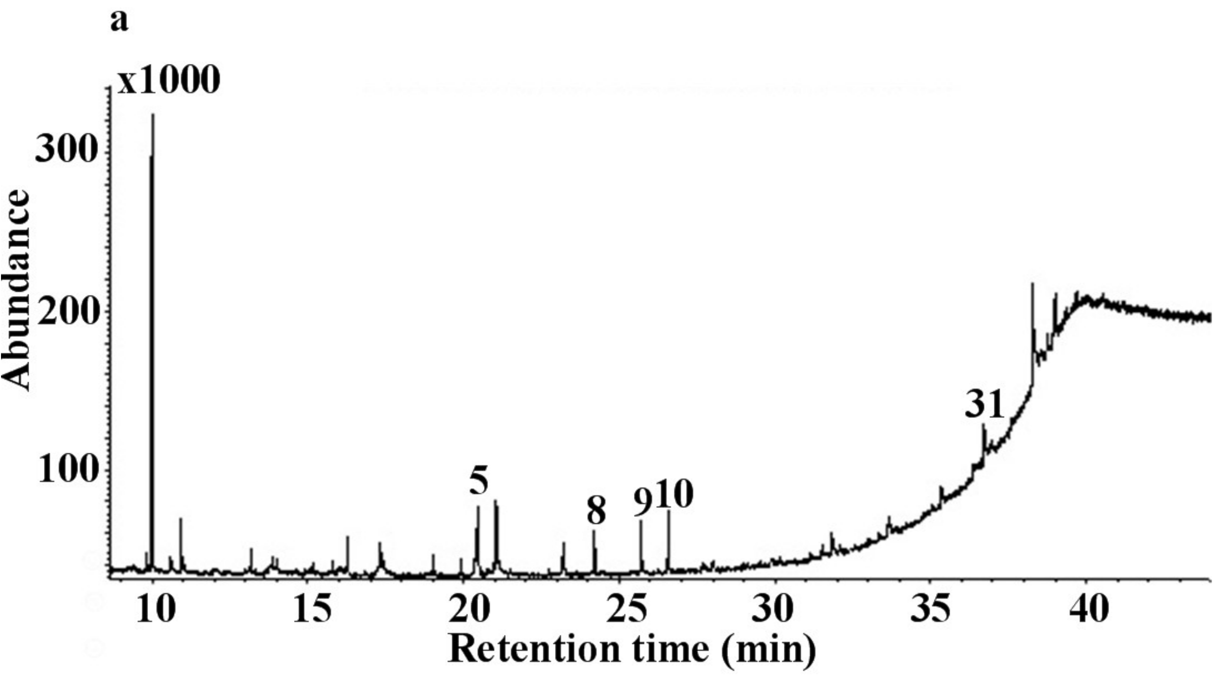


Figure 2



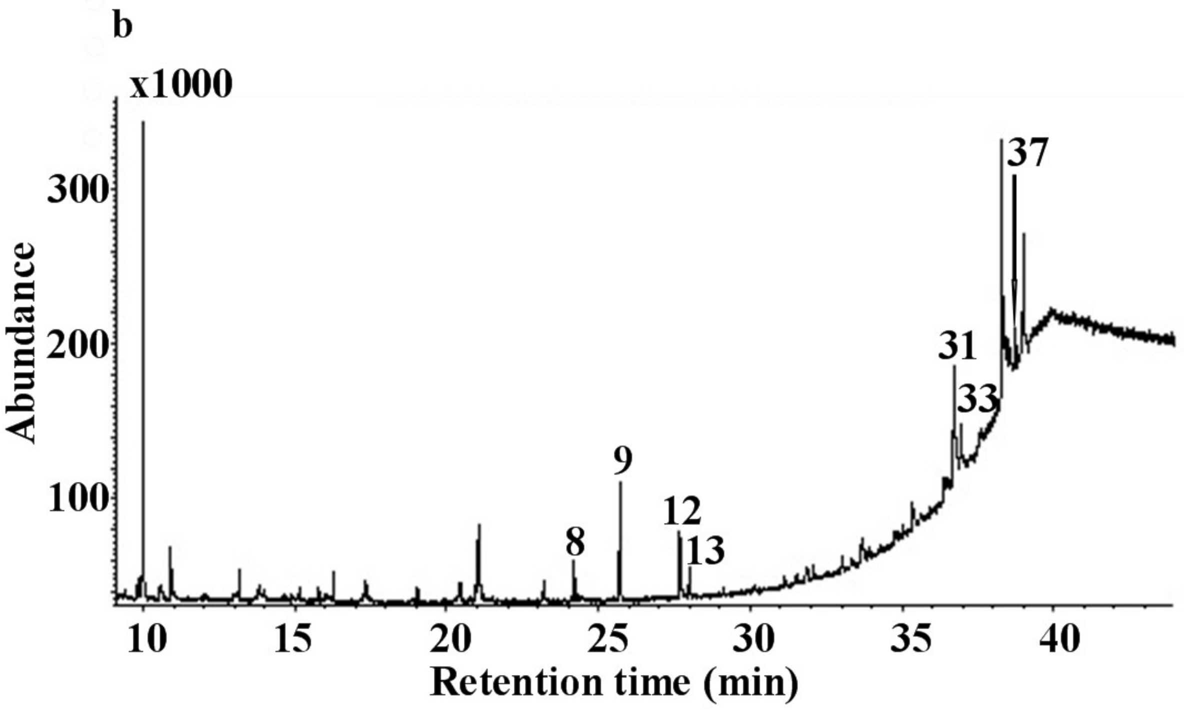
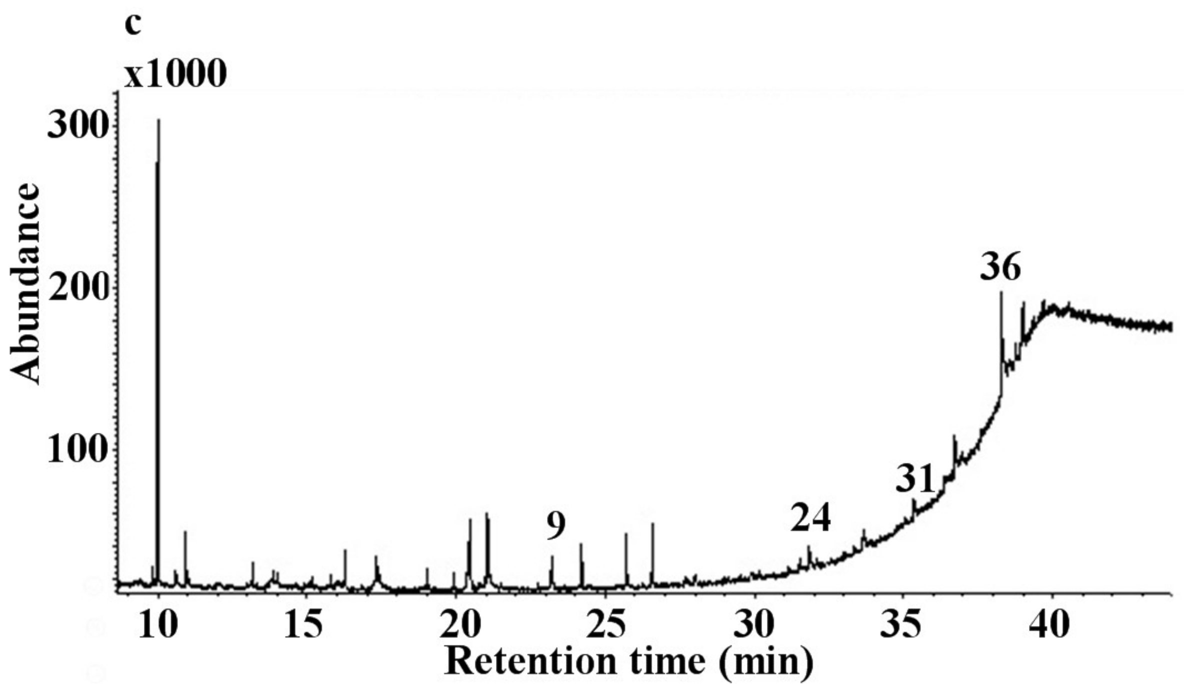


Figure 2 continued



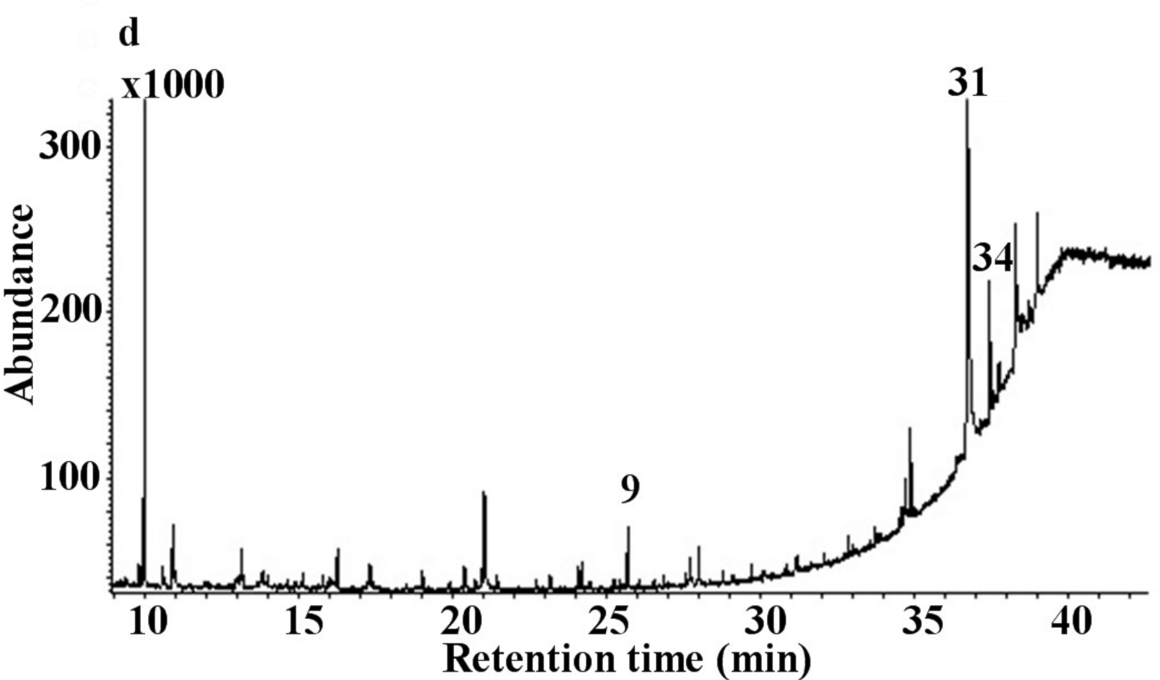
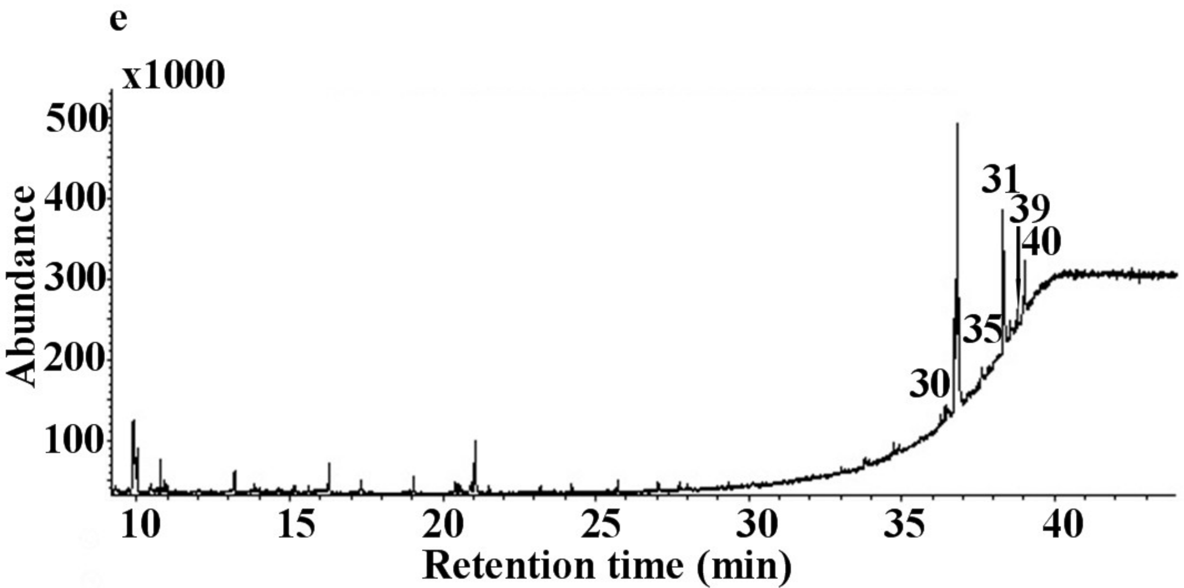


Figure 2 continued



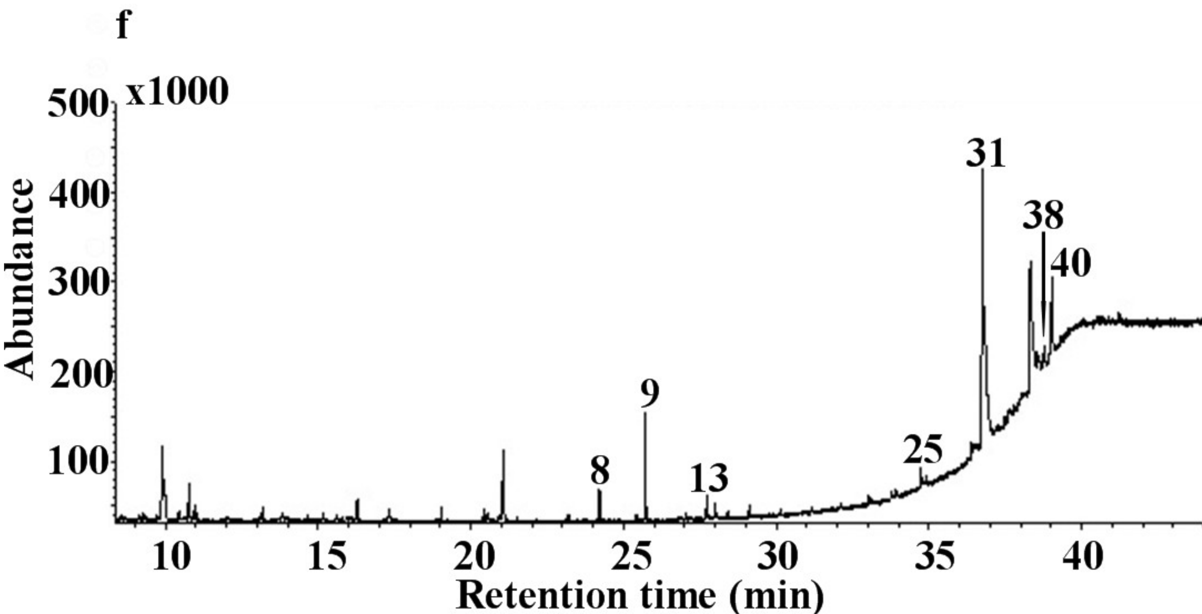
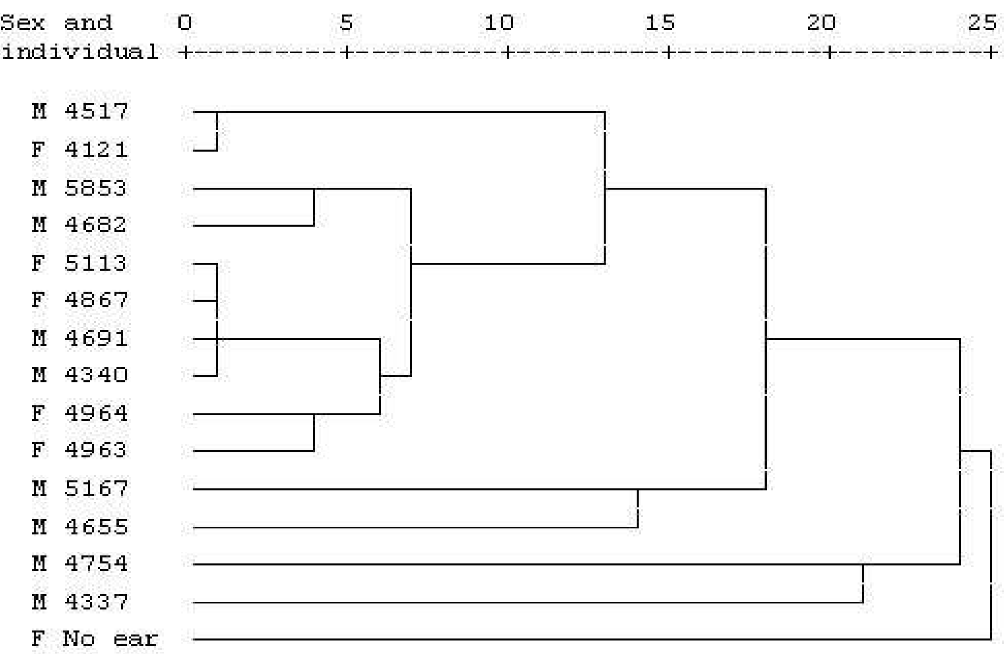


Figure 3

a



b

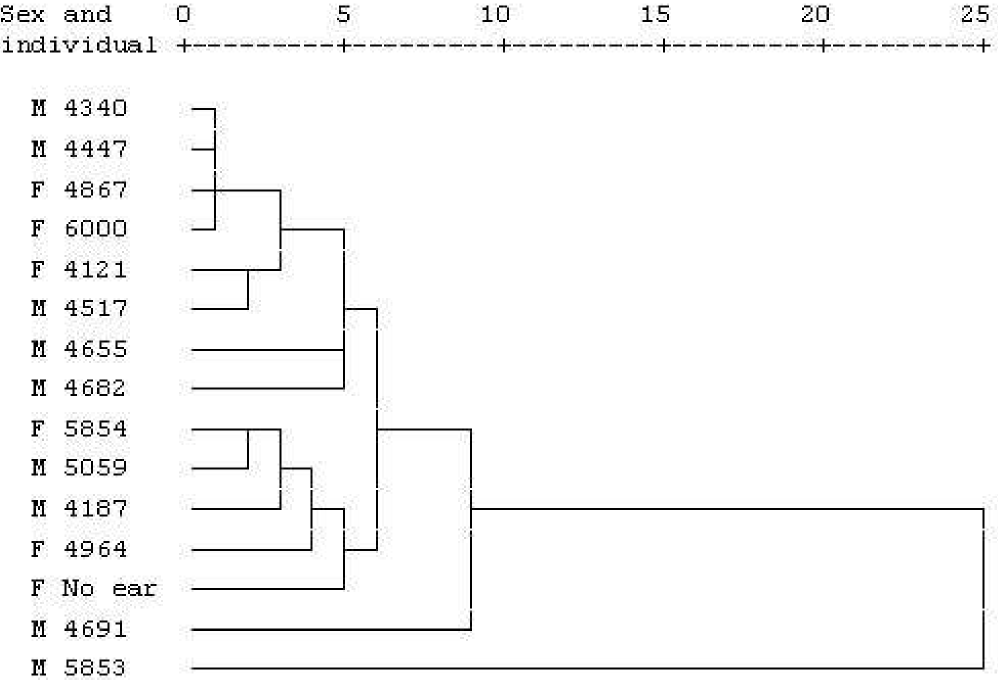
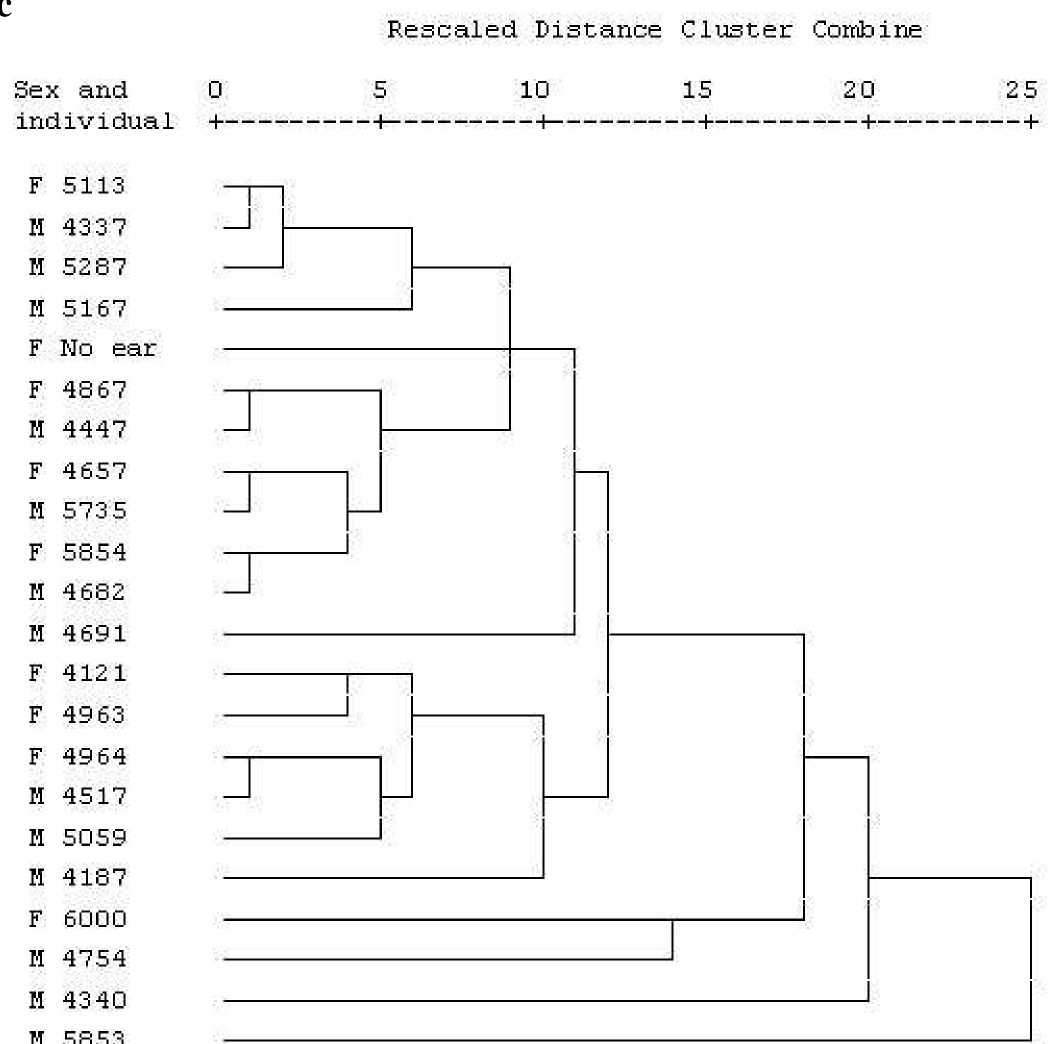


Figure 3 continued

c



Paper 2

Do anal gland secretion and feces of brown bear (*Ursus arctos*) code for sex?

Kristian Ingdal and Bård Andreas Lassen

To be submitted to Journal of Chemical Ecology

DO ANAL GLAND SECRETION AND FECES OF BROWN BEAR (*URSUS ARCTOS*) CODE FOR SEX?

KRISTIAN INGDAL[1,*] and BÅRD ANDREAS LASSEN[1]

[1]*Faculty of Art and Sciences*

Department of Environmental and Health Studies

Telemark University College

N-3800 Bø i Telemark, Norway

Abstract—Use of scent communication appears in most carnivores, and many of them use anal gland secretion (AGS) and feces as sources of olfactory information. The scent can contain various types of information, and sex related cues are found in many species. Little is, however, known whether the brown bear (*Ursus arctos*) use AGS and feces in intra-specific communication. We investigated AGS and feces from 29 free ranging bears by gas chromatography-mass spectrometry. We found 90 compounds (males = 74, females = 59) in AGS and 138 compounds (males = 96, females = 123) in feces. Our results supported the hypothesis that AGS and feces code for information about sex. The predictions that male and female AGS differ in gas chromatograms (GC), in analog coding and in color, were all supported. So was also the prediction of sex differences in GCs from feces. The predictions of sex differences in number of detected compounds and in digital coding of AGS were not supported. Neither were the predictions of sex differences in number of detected compounds and digital and analog coding of feces. Both AGS and feces code for sex, and might be used by bears for sex determination and in scent marking.

Key Words—Brown bear, *Ursus arctos*, anal gland secretion, feces, gas chromatography-mass spectrometry, digital and analog coding, partial least squares regression.

*To whom correspondence should be addressed. E-mail: ingdal2003@hotmail.com

INTRODUCTION

Olfactory communication plays an important role in mammalian social and reproductive behavior, and many species use scent to send out information about individual identity, reproductive status, territory boundaries and kin relations (Wyatt, 2003; Müller-Schwarze, 2006). The odor is often released through feces, urine and different kinds of scent glands, and contains a mixture of chemical compounds with varying volatility (Brown and Macdonald, 1985; Wyatt, 2003).

Use of olfactory cues appears in most carnivores (Macdonald, 1985; Halpin, 1986), and has been investigated in several species of mustelids and canids (e.g. Bowen and Cowan, 1980; Roper et al., 1986; Hutchings and White, 2000; Barja et al., 2005). Common for many of the carnivores is that they use anal gland secretion (AGS) for scent marking in order to send out olfactory information (Macdonald, 1985). The anal glands are normally situated in the cloaca area, and the secretion is discharged through ducts just internal to the anus (Dunstone, 1993). The gland secretion might be deposit separately (Asa et al., 1985; Dunstone, 1993), but many species deposit AGS on the feces when defecating (Macdonald, 1980; Gorman and Trowbridge, 1989). The secretion can differ in color, and Rosell and Sun (1999) and Buesching et al. (2001) found that the color might give out information about the donor.

Few studies have investigated AGS within large and rare carnivores, but studies of captive giant pandas (*Ailuropoda melanoleuca*) have reported that they posses large anal

glands that secrete a waxy substance (Schaller et al., 1985). There have been no studies of AGS in the other seven (Breiter, 2008) bear species, and it is unclear whether they posses anal glands or not. Pocock (1921) reported that he had found greatly reduced anal sacs in the American black bear (*Ursus americanus*), but he did not find them in the brown bear (*U. arctos*). His findings were supported by Dyce et al. (1996) who stated that bears do not possess anal glands. However, Landa and Tømmerås (1997) used AGS from a brown bear in a bioassay, without describing how they obtained the sample. Even though this indicates that brown bears posses anal glands, no anatomical details exist on the structures of the glands.

Swaisgood et al. (1999) suggested that captive giant pandas use their anal glands in scent marking behavior. The AGS is left as social signals within the habitat, and the pandas can determine the sex and age of the AGS donor and discriminate between different individuals (Swaisgood et al., 1999; Yuan et al., 2004). Chemical investigations supported these findings by revealing that AGS compounds coded for sex (Yuan et al., 2004; Zhang et al., 2008). Sex differences have also been revealed by chemical studies of AGS from other carnivores (e.g. Zhang et al., 2002; Zhang et al., 2005), and it is therefore likely to find this in brown bears as well.

Feces are also used in mammalian scent marking, and different messages are conveyed by the odor (Macdonald, 1980). Otters (*Lutra lutra*) use spraints of feces to communicate the use of key resources like food or shelter, while Iberian wolves (*Canis lupus signatus*) and badgers (*Meles meles*) use feces to mark their territory boundaries (Hutchings and White, 2000; Barja et al., 2005). Badgers often leave their feces in latrines for intra-specific communication (Buesching and Macdonald, 2001), and this behavior is also found within bears. Yoganand et al. (in press) reported that the sloth bear (*Melursus ursinus)* sometimes left their feces at the base of marking trees, while Mealey (1980) observed that grizzly bears (*U. a. horribilis*) left some feces in latrines. Kilham and Gray (2002) suggested

that also the American black bears use feces for sending out olfactory messages. All these findings imply that bear feces might contain information used in social communication. This is supported by Tschanz et al. (1970) who described how captive brown bears could distinguish the sex of another bear from feces. However, it is still unclear whether this was a result of sex related compounds in fecal odor or in the AGS deposited on the feces.

Even though sex differences in fecal odor are found in other carnivores, these differences might be caused by deposited AGS. However, investigation of domestic dogs (*C. familiaris*) showed that feces without AGS contained information about sex (Dunbar, 1977). Barja et al. (in press) found that Iberian wolf feces contained sex and stress hormones, and it is therefore likely to assume that also brown bear feces might contain sexual related cues. Despite that several chemical studies have investigated AGS, the knowledge about the chemical compounds in feces is limited. So far, no studies have investigated the chemical composition of feces within the Ursidae.

The main reason why little is known about olfactory communication in bears is probably that they are rare and hard to obtain fresh samples from. They are difficult to capture, and the access to study animals is low. Because of this has only scents from captive animals been used in former chemical studies of bears (e.g. Swaisgood et al., 1999, Yuan et al., 2004; Zhang et al., 2008). This might give results that are not representative for wild animals (Müller-Schwarze, 2001). Our study is the first to investigate AGS and feces from free ranging bears by chemical analyses.

Former chemical studies of mammalian scent have used various methods for investigating sex differences (Table 1). The methods use different characteristics of the scent to check for sex differences, and to date, no species has been examined by all these methods. In this study we used five different methods to investigate sex differences in AGS and feces.

We hypothesized that that brown bear AGS and feces code for sex, and predicted that AGS and feces have sex differences in gas chromatograms (GC), in number of compounds, and in digital (absence/presence of compounds) and analog (relative abundance of shared compounds) coding of chemical compounds. We also predicted that the color of the AGS differed between males and females.

METHODS AND MATERIALS

Study Area and Animals. The study was conducted in Dalarna and Gävleborg counties in south-central Sweden (61°N, 14°E) (Figure 1). The counties are within the southern part of the Scandinavian brown bear population (Sahlén et al., 2007), with a bear density at ~30 bears/ 1.000 km^2 (Zedrosser et al., 2006). The area is dominated by forest, and the most common species is scots pine (*Pinus sylvestris*). Other common species are Norway spruce *(Picea abies)*, birch *(Betula spp.)*, aspen *(Populus tremula)* and the planted lodgepole pine (*Pinus contorta)*. The forestry is intensive in the area, and together with roads, mires and lakes it makes a patchy landscape.

A total of 29 free ranging bears were used in the study. The bears were darted and immobilized from a helicopter, using a remote drug delivery system (Dan-Inject®) with a combination of tiletamine/zolazepam and medetomidine (Arnemo and Fahlman, 2007). The animals were sexed by checking the genitals. Age determination of bears that were not captured as yearlings, was based on cementum annuli in the premolar tooth root (Matson et al., 1993). The first premolar tooth in the upper jaw was analyzed at Matson`s Laboratory (Milltown, MT, USA). Male age differed from two to 17 years while female age differed from three to 18 years (Table 2). All animals were equipped with a global positioning system (GPS) collar including a global system for mobile communication (GSM) lateral modem (VECTRONIC Aerospace GmbH, Berlin, Germany). The collar stored a universal transverse

mercator (UTM) position every 30 min, and these positions were downloaded to a database every morning.

AGS and Feces Collection. We collected AGS samples from 17 bears (male N = 5, female N = 12) during two periods prior to the mating season (April 18 - May 18, 2007 and April 6 - 30, 2008). The immobilized bear was lying on the side when we squeezed out AGS by hand by putting a pressure on each anal pocket separately. All samples were put on labeled and dated 40 ml glass vials with teflon lined cap (Lab Safety Supply®, WI 53547-1368, USA).

We collected 17 samples of feces (male N = 4, female N = 13) between June 1-12, 2007. To find feces we visited daybeds were the bears rest during the day. These daybeds (clusters of positions between 06.00 am to 06.00 pm) were identified by visual cluster analyses of UTM coordinates from the database. The analyses were performed in a Geographic Information System platform (ArcView® GIS 3.2). We walked into the daybed locations and looked for feces within a radius of 30 meters (Rauset, 2006). We only collected feces from daybed locations with only one daybed. If we found two or more fresh daybeds, the feces in the area could theoretically be from an unknown bear in company with the GPS-collared one. As a compromise between getting relatively fresh samples and avoiding disturbance of the bears, we only collected samples that were 12-30 hr old. We put the feces in labeled and dated 500 ml glass vials with teflon lined cap (Lab Safety Supply®, WI 53547-1368, USA).

We always used latex gloves during collection of AGS and feces, to avoid contamination by human scent. All AGS and feces were immediately placed on ice after collection. The samples were frozen at -20°C within 12 hr of collection, and kept frozen until analysis. We transported the samples in a cooling box to the laboratory in Norway, and they all kept frozen during transport.

AGS Color Comparison. We used the natural color system (NCS) (Scandinavian Colour Institute, Stockholm, Sweden) to check for sex difference in the color of male and female AGS (Rosell and Sun, 1999). The color of the secretion was ranked by eye into 12 categories from 1 = light to 12 = very dark (Buesching et al., 2001).

Chemical Sample Preparation. We performed a pre-study with different kinds of solvents, and found that toluene-methanol 3:1 extracted most compounds from the AGS. This solvent was also successfully used by Rosell and Sundsdal (2001) for detecting compounds in the AGS of Eurasian beaver (*Castor fiber*). We transferred 0.1 gram of AGS into a small glass test tube by using a sterile needle. We added 1 ml of toluene-methanol 3:1, and vortexed the solution for 15 sec. The compounds were extracted for 2 hr in room temperature before we centrifuged the sample for 3 min at 3500 rpm. We covered the glass test tube with aluminum foil during extraction and centrifugation to avoid loss of volatile compounds. After centrifugation, we pipetted the particle free solution into a GC-vial. This solution was used in the analyses.

We extracted compounds from feces by using solid-phase microextraction (SPME) (Pawliszyn, 1999). This method is previously used to extract compounds in scent from Asian elephant (Dehnhard et al., 2003). Different types of SPME fibers were tested in advance of the study, and the 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Inc., Bellefonte, Pennsylvania, USA) extracted most compounds. For extraction of chemical compounds in feces, we weighted 100 grams into a 250 ml glass beaker, which was covered with aluminum foil to prevent volatile compounds to emerge. This headspace technique extracted only the volatile compounds.

We used the SPME fiber in a headspace technique, meaning that the fiber was inserted through the aluminum foil and kept in the air above the feces for 10 minutes. The feces were heated up to 40°C to release more volatile compounds. Prior to use, all laboratory equipment

were washed properly, cleaned in acetone and baked for 12 hr at 200 °C. This was done to avoid contamination of the samples.

Chemical Analysis. In all analyses we used a Hewlett-Packard (HP) 6890 Series II gas chromatograph equipped with a non-polar HP-5 MS 5% phenyl-methyl-siloxane column (30.0 m long x 0.25 mm ID x 0.25 μm film thickness) connected to a HP 5973 Series mass spectrometer detector with a split/splitless inlet used in the splitless mode. We used helium as the carrier gas at a constant flow of 1.0 ml/min, and the injection port temperature was set at 270°C. Purge flow to split vent was 49.8 ml/min@1.00 min. The instrument was regularly calibrated to detect possible changes in the sensitivity of the instrument. This prevented unstable conditions during the work.

We injected 1 μl of the particle free AGS solution into the gas chromatograph-mass spectrometer (GC-MS) by using an auto-injection system (Agilent 7683 Series Injector). 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. The initial oven temperature was set to 55°C for 2 minutes, and than increased 6°C/min to 310°C, which was maintained for 5 min. The entire run lasted for 49.50 min. The first 8 min were eliminated for further analysis because all peaks in this interval stemmed from either the solvent or the column.

For analyzing the feces, the SPME fiber with extracted compounds was inserted manually into the GC-MS equipped with a HP single taper liner (0.75 mm inner diameter). The initial oven temperature was set to 40°C for 5 minutes, and than increased 12°C/min to 300°C. The entire run lasted for 26.67 min. Before every run, the fiber was conditioned for one hour in the injection port to avoid contamination of previous samples. We ran control samples before, in the middle and after all the AGS and feces 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. This was done by a computer-aided compound search. Structures of unidentified compounds were added in a new library, which we included in the computer search. The new compounds could then be recognized in different samples by comparing structures and retention time. The mass spectra from the GC peaks and from the library were visually compared to see if the suggestions from the computer were reasonable. A positive identification of the compounds through known standards was not conducted, because this was not the focus of this study.

The area of every peak was found by a computer-aided integration. We set the threshold to 17.0 for the AGS and 18.0 for the feces to avoid integration of peaks which stemmed 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.

Statistics. Scent can communicate information trough a mixed composition of chemicals which can be investigated by looking at GC. In order to check for sex differences in chemical composition of AGS and feces, we compared GCs by using visual inspection. We also used partial least squares (PLS1) regression formerly used by Rosell and Steifetten (2004). PLS1 is a multivariate calibration method that uses information in Y-matrix actively to find the Y-relevant structure in X-matrix (Esbensen, 2002) (for details, see Rosell and Steifetten, 2004). As a basis for comparison, the abundance was measured for every time unit (165 time units/min) on the retention scale of the GC. The measured values formed two GC-matrixes of X-variables (7012 time measurements in the AGS, and 4388 in the feces) and Y-variables (sex of donors of 17 AGS samples and 17 feces samples). PLS1 was appropriate because the X-variables were strongly intercorrelated and the number of variables was greater than the number of samples (Wold et al., 1983). All values were scaled by mean

normalization and standard normal variate (SNV) to minimize the effect of considerable variation in abundance between the samples. Due to low sample size, we used leverage correction to estimate the prediction residuals. This might give an optimistic result (Esbensen, 2002). The PLS1 method extracts a small number of PLS1 components (PCs) which represent the relevant latent dimensions of the model. We use the values of validated R-square and root mean square error of prediction (RMSEP) to evaluate the results. Validated R-square tells about the predictive ability of the model, and the closer to 1, the better. RMSEP is a measurement of the average difference between predicted and measured response values, at the prediction or validation stage. A value closer to 0 the better. The statistical software used was The Unscrambler 9.7 (CAMO Software AS).

Olfactory information can also be sent out through digital and analog coding of chemical compounds in the scent (Sun and Müller-Schwarze, 1998a, b). This method is formerly used together with analysis by gas chromatography-mass spectrometry for revealing sex differences in composition of carnivore scent (Table 1). We used this idea of coding when investigating sex differences in detected compounds in AGS and feces.

We encoded the tentatively identified compounds by 0 (absent) and 1 (present) for all samples, and these data formed two digital matrixes of X-variables (detected compounds) and Y-variables (individuals). From the matrixes we could investigate the number of detected compounds within individuals and sex. Sex differences in number of detected compounds were analyzed by using the Mann-Whitney *U* test (Zar, 1998). A compound was defined to be sex specific if found in all males or females (Andersen and Vulpius, 1999). To check for sex difference in digital composition of male and female AGS and feces, the digital matrixes was used in a hierarchical cluster analysis with squared Euclidean distance (Yuan et al., 2004). We used cluster analysis because the matrixes had more variables (number of compounds) than

observations (number of individuals), and therefore canonical discriminant analysis was unsuitable (Johnson and Wichern, 1992).

We checked for sex differences in analog coding of AGS and feces by using relative abundance of each compound in quantitative analyses. Mann-Whitney *U* test was used to investigate the difference between the sexes. We also used Mann-Whitney *U* test to compare the color of the AGS from males and females. Non parametric test were used in all statistics because our data did not fit the assumption of normal distribution and homogeneity of variance for parametric analysis (Zar, 1998).

We used SPSS for Windows (version 15.0; SPSS inc. 1999) for all statistical analysis. The significant level was set to $P < 0.05$, and we defined a *P*-value between 0.05 - 0.1 as marginal significant (see Zhang et al., 2008).

RESULTS

Anal Gland Description. The brown bear possessed two-paired anal glands, which were located on each side of the anus (Figure 2). We were no able to collect AGS from all animals, as some of the anal glands seemed to be empty. The secretion had a clayey substance with an unpleasant smell. No obvious sex difference was detected by the human nose. The color of the AGS differed from nearly black to light gray (Table 3), and the male AGS showed a significantly darker color than the female AGS (Mann-Whitney *U* test, $U = 7.5$, $N_{male} = 5$, $N_{female} = 11$, $P = 0.02$).

GC Comparison. We found no clear sex difference in chemical composition of AGS through visual inspection of GCs of male and female brown bears (Figure 3a and b). However, a tendency was present. Four compounds seemed to have higher peaks in female than in male brown bears, and one compound seemed to have higher abundance in male than

in female brown bears. We found sex differences in the feces through visual inspection of the GCs.

The comparison of male and female GCs by PLS1 showed a sex difference in both AGS and feces. Despite some overlap, the PLS1 score plots of AGS (Figure 4) and feces (Figure 5) showed a clear tendency of male and female separation. Of the total variation within the AGS GCs, PC 1 explained 39% of the X-variance and 30% of the Y-variance, while PC 2 explained 20% of the X-variance and 38% of the Y-variance. Validated R-square = 0.82, and RMSEP = 0.20. Of the total variation within all feces GCs PC 1 explained 11% of the X-variance and 46% of the Y-variance, while PC 2 explained 28% of the X–variance and 17% of the Y-variance. Validated R-square = 0.88, and RMSEP = 0.14.

Digital Coding. We found 90 compounds in brown bear AGS. In general these compounds were classified as fatty acids, hydrocarbons and different steroids. Of the compounds where we could determine the molecular weight (MW), 68% were above 300 MW (Table 4). We found 138 compounds in brown bear feces. The compounds in the feces were classified as alcohols, sulfur compounds, organic acids, esters and hydrocarbons, and 100% of the compounds that we could determine the MW of, was below 300 MW (Table 5).

In the AGS we found a total of 74 ($\overline{X} \pm SD = 38.00 \pm 8.87$) different compounds in males and 59 ($\overline{X} \pm SD = 29.14 \pm 7.78$) compounds in females. There were no sex difference in total number of detected compounds ($U = 6.5$, $N_{male} = 4$, $N_{female} = 7$, $P = 0.12$). Of the compounds identified in the AGS, no. 7, 10, 11, 16, 17, 19, 23, 24, 26, 30, 31, 33-35, 38, 40, 43, 46, 50, 53, 62, 65, 67, 72, 75, 81, 84 and 86-89 were only found in males, and compound no. 1-4, 6, 12, 22, 29, 32, 47, 54, 66, 68, 77, 82 and 90 were only found in females. Compound no. 27, 44, 48, 51, 57, 58, 60, 70, 73, 74, 76, 80 and 85 were shared by all males and females (Table 4).

In feces we found a total of 96 ($\bar{X}$ ± SD = 48.50 ± 12.77) different compounds in males and 123 ($\bar{X}$ ± SD = 40.54 ± 12.37) compounds in females, but no sex difference was found (U=15, N_{male}=4, N_{female}=13, P = 0.23). Of the compounds found in the feces, no. 5, 6, 9, 14, 19, 21, 24, 25, 40, 41, 56, 59, 61, 107 and 114 were only found in males, and no. 2, 13, 20, 29, 30, 34, 39, 45, 48, 49, 51, 54, 57, 60, 62-64, 71, 80, 81, 85, 88, 90, 96, 98, 100, 102-104, 106, 111, 115, 116, 120, 121, 125, 129, 130, 132, 134, 136 and 138 were only found in females. Compound no. 1, 69, 79, 89 and 109 were shared by all male and females (Table 5).

We did not find any sex specific compounds in either AGS or feces. We also failed to find any clear classification patterns between the sexes in AGS or feces by using hierarchical cluster analysis (Figure 6a and b). We also found great individual differences (Table 4 and 5).

Analog Coding. We found differences between male and female brown bears in the relative abundance of some of the shared compounds in the AGS. Female bears had a significant higher abundance than male bears in four compounds: 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), and the male bears had a marginally significant higher relative peak area in compound no. 76 (a steroid) (U = 4, P = 0.058) (Figure 7). There were no significant sex differences in the relative peak area of shared compounds in the feces (all $P > 0.43$).

DISCUSSION

This study is the first to investigate anal glands and the chemical composition of AGS and feces in brown bears. Our results supported the hypothesis that AGS and feces code for information about sex. The predictions that male and female AGS differ in GCs, in analog coding and in color, were all supported. So was also the prediction of sex differences in GCs from feces. The predictions of sex differences in number of detected compounds and in digital

coding of AGS were not supported. The prediction of sex differences in number of detected compounds and digital and analog coding of feces were either not supported.

The anal glands of brown bears seem to be very similar to the anal sacs of the domestic dog, where the sacs are located between the external and internal sphincter muscles (Dyce et al., 1996). These muscles help emptying the content in the intestine, and the secretion drains trough a single duct to an opening near the anus (Dyce et al., 1996). We were not able to collect AGS from all the brown bears and this problem increased closer to and in the mating season (Sven Brunberg, personal communication). This can imply that the anal glands contain less secretion because of more frequently use in the mating season. The non-social bears are more likely to meet during this period, and may use the AGS more rapidly to communicate their presence. Increased marking activity in the breading season is also reported in American black bear and giant panda. Burst and Pelton (1983) reported that American black bears rubbed marking trees more frequently in the mating season and Schaller et al. (1985) stated that the giant panda increased the use of AGS during this period.

We revealed sex differences in color of AGS, and male secretion was in general darker than female secretion. Sliwa (1996) found a comparable pattern is in aardwolves (*Proteles cristatus*). In contrast to North American beaver (*C. canadensis*) (Schulte et al., 1995), aardwolves (Sliwa, 1996), Eurasian beaver (Rosell and Sun, 1999) and badger (Buesching et al., 2001), the brown bear male and female AGS had some overlap in color, and the sex differences were not 100% consistent. The color of AGS should therefore not be used for sex determination of the donor.

In contrast to Rosell and Sundsdal (2001), we could not find any clear sex related GC patterns in the AGS or feces through the visual inspection. This type of comparison is nevertheless a limited method for comparing GCs. However, the PLS1 analysis showed sex differences in chemical composition of GC in both AGS and feces. This implies that sex

might be coded through a specific mix of several compounds (Albone, 1984) where both the presence and amount of compounds are important. Our result is in accordance to Rosell and Steifetten (2002) who found that AGS in both Eurasian and North American beaver code for species and sex through a complex mixture of chemical compounds.

Like in other carnivores, we found sex differences in analog coding of brown bear AGS (Table 1). Similar coding is also found for nine compounds in AGS of the giant panda (Yuan et al., 2004). The major differences in the panda were found in four steroids, but we can not say whether this is the same four steroids as in brown bears. We suggest that the relative abundance of these steroids plays an important role in gender discrimination in brown bears. However, this should be investigated further by chemical and behavioral studies.

The total number of compounds detected in the AGS is relatively high compared to scent marking carnivores like steppe polecat (*Mustela eversmanni*), Siberian weasel (*M. sibirica*) and domestic dog (Table 1). Still, the total number of compound we found in the AGS, is this quite similar to what Yuan et al. (2004) found in AGS from giant panda (Table 1). This amount of compounds might code for a wealth of information (Albone, 1984), and implies that brown bear might use AGS in scent communication. Many of the compounds we identified in AGS were fatty acids, fatty acid-esters, steroids and hydrocarbons, and this are also found in wolves (*Canis lupus*) (Raymer et al., 1985), domestic dogs (Natynczuk et al., 1989), giant pandas (Yuan et al., 2004) and wolverines (*Gulo gulo)* (Wood et al., 2005). We identified 138 compounds in the brown bear feces, and among these did we found organic acids, heptanal, phenol and nonene which are also present in domestic dog (Arnould et al., 1998). Even though we used the headspace SPME-technique to avoid compounds from the diet, many compounds are probably still dietary derivatives. This makes it more difficult to reveal sex differences in the scent (Burger, 2005). In contrast to Eurasian beaver, we did not find any sexual dimorphism in number of compounds in AGS (Rosell og Sundsdal, 2001).

Neither did we find similar differences in feces. This implies that bears can not use the number of compounds to distinguish between individuals of different sex.

The digital coding of AGS and feces revealed great individual differences. Especially in feces it is likely that this is a result of diet. The differences might also be a result of different individual information in the scent. However, we did not find any sex specific compounds in AGS nor feces, but the result would have been different if we had used the same definition as Zhang et al. (2003) used for 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 would compound no. 90 in AGS be defined as sex-specic. However, we found it more biological right to follow the definition of Andersen and Vulpius (1999).

The hierarchical cluster analyses failed to show a clear grouping of sex in both AGS and feces. Still, 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 subadult rather than sex (Liu et al., 2006). In this study, we did not have enough subadults ($\leq$ 3 years) to investigate grouping patterns between age classes in brown bears (Table 2).

Since the brown bears have large estimated home ranges (male = 1055km^2, female = 217 km^2) (Dahle and Swenson, 2003), they have a comprehensive area to scent mark. These marks would have to last for a long time in order to be an effective form of communication. Compounds of low volatility are therefore useful chemical signals for long-lasting or delayed communication because infrequent renewal could save a substantial energy associated with patrol (Yuan et al., 2004). Compounds with MW > 300, the upper limit for airborne pheromones (Wilson, 1963; Bradbury and Vehrencamp, 1998), are well suitable for a large home range. Our results showed that 68% of the compounds detected in the AGS had a MW

above 300. This supports that AGS might be used in long-lasting or delayed communication, just like in the giant panda (Yuan et al., 2004).

The detected compounds in feces had a generally lower molecular weight than what we found in AGS. 100% of the compounds had MW < 300, but this is probably because the SPME-fiber extracts mainly volatile compounds with MW < 275. Our findings of sex differences in GC imply that feces have a function in chemical communication, despite of low MW. This is supported by Arnould et al. (1998) who found that domestic dogs could determine sex of donors of the feces, even if AGS was not deposited. Additionally, Barja et al. (in press) found that scats that were presumed to have a marking role had significantly higher levels of sex hormones than those that did not. This supports our findings of sex differences in feces.

Carnivores like the mink (*Mustela vison*) (Macdonald, 1985), wolf (Asa et al., 1985), domestic dog (Dyce et al., 1996) and coyote (*Canis latrans*) (Tegt et al., 2004) leave feces with deposited AGS in or at the border of their home range or territories. Even though nothing is known about how brown bears use their AGS, it is likely to assume that also they are depositing the secretion while defecating. This means that the feces samples might be contaminated with AGS. According to Macdonald (1980) and Gorman and Trowbridge (1989) almost all the carnivore families use only small volumes (tokens) of AGS when depositing on the feces and this implies that the chances of getting AGS in the feces analyses are relatively small. Especially since we only uses 100 grams of each feces sample. Asa et al. (1985) showed that AGS from wolves were present in less than 10% of the scent marking scats, and if bears use AGS on the same amount of feces, the chances of getting contaminated feces is even smaller.

GPS data from free ranging brown bears has revealed some interesting matters that imply that the bears somehow can recognize each other. Related females overlap more in

home range than unrelated females, which indicate that related are more tolerant of each other than unrelated females (Støen et al., 2005). Zedrosser et al. (2006) reported that young male bears disperse from their natal areas to avoid intra-sexual mate competition with older bears. This implies that brown bears somehow can discriminate between individuals. It is likely to assume that brown bears might use scent for this purpose, just like other species such as giant panda (Swaisgood, 1999), steppe polecat (Zhang et al., 2002) and coyote (Tegt et al., 2004).

Roth (1980) used feces as an indicator of brown bear abundance in an area, and bears themselves could probably do the same thing. According to the scent matching hypothesis intruders learn the odor of the home range owner from the scent marks in the area, so that they can recognize the owner and avoid fights they are likely to lose (Wyatt, 2003). AGS and feces might have a function like this among the bears.

This study has revealed that brown bear AGS and feces code for information about sex, and might be used for sex determination and scent marking. The findings might be important for the understanding of the brown bear social life, but further chemical and behavioral studies are needed.

Acknowledgments— We thank the entire research personnel in the Scandinavian Brown Bear Research Project. It was very inspiring to work on such a well established project. A special thank to project leader Jon Swenson for giving us the opportunity to participate in the project, Andreas Zedrosser for valuable assistance in field and comments on earlier drafts, and to field work coordinator Sven Brunberg. We also want to thank Bjørn Steen for assisting us during GC-MS analyses and Frank Rosell for initiating this study, assistance in the field and comments on earlier drafts of the manuscripts. The study was supported financially by the conservation departments in Finnmark, Nord-Trøndelag, and Hedmark counties.

REFERENCES

ALBONE, E. S. 1984. Mammalian Semiochemistry: The Investigation of Chemical Signals Between Mammals. John Wiley, Chicester.

ANDERSEN, K. F. and VULPIUS, T. 1999. Urinary volatile constituents of the lion, *Panthera leo*. *Chem. Senses* 24:179-189.

ARNEMO, J. M. and FAHLMAN, Å. 2007. Biomedical protocols for free-ranging brown bears, gray wolves, wolverines and lynx. Norwegian School of Veterinary Science, Tromsø, Norway.

ARNOULD, C., MALOSSE, C., SIGNORET, J., and DESCOINS, C. 1998. Which chemical constituents form dog feces are involved it its food repellent effect in cheep? *J. Chem. Ecol*. 24:559-576.

ASA, C. S., PETERSON, E. K., SEAL, U. S., and MECH, D. L. 1985. Deposition of anal sac secretion by captive wolves (*Canis lupus*). *J. Mammal*. 66:89-93.

BARJA, I., MIGUEL, F. J., and BARCENA, F. 2005. Faecal marking behaviour of Iberian wolf in different zones of their territory. *Folia Zool.* 54:21-29.

BARJA, I., SILVÁN, G., and ILLERA, J. C. in press. Relationships between sex and stress hormone levels in feaces and marking behavior in a wild population of Iberian wolves (*Canis lupus signatus*). *J. Chem. Ecol.*

BOWEN, W. D. and COWAN, I. M. 1980. Scent marking in coyotes. *Can. J. Zool*. 58:473-480.

BRADBURY, J. W. and VEHRENCAMP, S. L. 1998. Principles of Animal Communication. Sinauer Associates, Sunderland, Massachusetts.

BRADSHAW, A., BECKMANN, M., STEVENS, R., and SLATER, F. 2001. Anal scent gland secretion of the European otter (*Lutra lutra),* pp. 313-319*,* in A. Marchlewska-Koj, J. J. Lepri, and D. Müller-Schwarze (eds.). Chemical Signals in Vertebrates 9. Kluwer Academic/ Plenum Publishers, New York.

BREITER, M. 2008. Bears, a Year in the Life. A & C Black, London.

BROWN, R. E. and MACDONALD, D. W. 1985. Social Odours in Mammals. Oxford, Clarendon.

BUESCHING, C. D. and MACDONALD, D. W. 2001. Scent-marking behaviour of the European badger (*Meles meles*): Resource defence or individual advertisement?,pp. 321-328, in A. Marchlewska-Koj, J. J. Lepri, and D. Müller-Schwarze (eds.). Chemical Signals in Vertebrates 9. Kluwer Academic / Plenum Publisher, New York.

BUESCHING, C. D., NEWMAN, C., and MACDONALD, D. W. 2001. Variations in colour and volume of the subcaudal gland secretion of badgers (*Meles meles*) in relation to sex, season and individual-specific parameters. *Mamm. Biol.* 67:147-156.

BURGER, B. V. 2005. Mammalian semiochemicals. *Top. Curr. Chem.* 240:231-278.

BURST, T. L. and PELTON, M. R. 1983. Black bear mark trees in the Smoky Mountains. *Int. Conf. Bear Res. Manage.* 5:45-53.

DAHLE, B. and SWENSON, J. E. 2003. Home ranges in adult Scandinavian brown bears (*Ursus arctos)*: effect of mass, sex, reproductive category, population density and habitat type. *J. Zool.* 260:329-335.

DEHNHARD, M., HATT, J. M., EULENBERGER, K., OCHS, A., and STRAUSS, G. 2003. Headspace solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS) for the determination of 5ά-androst-2-en-17-one and -17 ß -ol in the female Asian elephant: application for reproductive monitoring and prediction of parturition. *J. Steroid Biochem. Mol. Biol.* 84:383-391.

DUNBAR, I. F. 1977. Olfactory preferences in dogs: The response of male and female beagles to conspecific odors. *Behav. Biol.* 20:471-481.

DUNSTONE, F. 1993. The Mink. T & A D Poyser Ltd., London.

DYCE, K. M., SACK, W. O., and WENSING, C. J. G. 1996. Textbook of Veterinary Anatomy. W. B Saunders Company, Philadelphia.

ESBENSEN, K. E. 2002. Multivariate Data Analysis - in Practice: an Introduction to Multivariate Data Analysis and Experimental design. 5th ed. Camo, Oslo.

GORMAN, M. L. and TROWBRIDGE, B. J. 1989. The role of odor in the social lives of carnivores, pp. 57-139, in J. L. Gittleman (ed.). Carnivore Behavior, Ecology and Evolution. Cornell University Press, New York.

HALPIN, Z. T. 1986. Individual odors among mammals: origins and functions. *Adv. Stud. Behav*. 16:39-70.

HUTCHINGS, M. R. and WHITE, P. C. L. 2000. Mustelids scent-markings in managed ecosystems: implications for population management. *Mammal. Rev*. 30:157-169.

JOHNSON, R. A. and WICHERN, D. W. 1992. Applied Multivariate Statistical Analysis. Prentice-Hall, New Jersey.

KILHAM, B. and GRAY, E. 2002. Among the Bears: Raising Orphan Cubs in the Wild. Henry Holt and Company, LLC, New York.

LANDA, A. and TØMMERÅS, B. Å. 1997. A test of aversive agents on wolverines. *J. Wildl. Manage*. 61:510-516.

LIU, D., YUAN, H., TIAN, H., WEI, R., ZHANG, G., SUN, L., WANG, L., and SUN, R. 2006. Do anogenital gland secretions of giant panda code for their sexual ability? *Chin. Sci. Bull*. 51:1986-1995.

MACDONALD, D. W. 1980. Patterns of scent marking with urine and faeces amongst carnivore communities. *Symp. Zool. Soc. Lond*. 45:107-139.

MACDONALD, D. W. 1985. The carnivores: order Carnivora, pp. 617-722, in R. E. Brown and D. W. Macdonald (eds.). Social Odours in Mammals. Oxford, Clarendon.

MATSON, G., DAELE, L. V., GOODWIN, E., AUMILLER, L., REYNOLDS, H. and HRISTIENKO, H. 1993. A laboratory manual for cementum age determination of Alaska brown bear first premolar teeth. Alaska department of fish and game division of wildlife conservation. Matson`s laboratory, Milltown, MT, USA.

MEALEY, S. P. 1980. The natural food habits of grizzly bears in Yellowstone National Park, 1973-74. *Int. Conf. Bear. Biol. Manage*. 4:281-292.

MÜLLER-SCHWARZE, D. 2001. From individuals to populations: Field studies as proving grounds for the role of chemical signals, pp. 1-10, in A. Marchlewska-Koj, J. J. Lepri, and D. Müller-Schwarze (eds.). Chemical Signals in Vertebrates 9. Kluwer Academic / Plenum Publisher, New York.

MÜLLER-SCHWARZE, D. 2006. Chemical Ecology of Vertebrates. Cambridge University Press, Cambridge.

NATYNCZUK, S., BRADSHAW, J. W. S., MACDONALD, D. W. 1989. Chemical constituents of the anal sacs of domestic dogs. *Biochem. Syst. Ecol.* 17:83-87.

PAWLISZYN, J. 1999. Applications of Solid Phase Microextraction. The Royal Society of Chemistry, Cambridge.

POCOCK, R. I. 1921. The external characters and classification of the Procyonidae. *Proc. Zool. Soc. Lond.* 27:389-422.

PRETI, G., MUETTERTIES, E. L., FURMAN, J. M., KENNELLY, J. J., JOHNS, B. E. 1976. Volatile constituents of dog (*Canis familiaris*) and coyote (*Canis latrans*) anal sacs. *J. Chem. Ecol.* 2:177-186.

RAUSET, G. R. 2006. Estimating individual kill rates on moose calves by brown bears based on GPS technology and GIS cluster analysis. M.Sc. thesis, The Norwegian University of Life Sciences (UMB).

RAYMER, J., WIESLER, D., NOVOTNY, M., ASA, C., SEAL, U. S., and MECH, L. D. 1985. Chemical investigations of wolf (*Canis lupus*) anal-sac secretion in relation to breeding season. *J. Chem. Ecol.* 11:593-608.

ROPER, T. J., SHEPERDSON, D. J., and DAVIES, J. M. 1986. Scent marking with feces and anal secretion in the European badger (*Meles meles*): Seasonal and spatial characteristics of latrine use in relation to territoriality. *Behaviour* 97:94-117.

ROSELL, F and STEIFETTEN, Ø. 2004 Subspecies discrimination in the Scandinavian beaver (*Castor fiber*): combining behavioral and chemical evidence. *Can. J. Zool.* 82:902-909.

ROSELL, F. and SUN, L. 1999. Use of anal gland secretion to distinguish the two beaver species *Castor canadensis* and *C. fiber*. *Wildl. Biol.* 5:119-123.

ROSELL, F. and SUNDSDAL, L. J. 2001. Odorant source used in Eurasian beaver territory marking. *J. Chem. Ecol*. 27:2471-2491.

ROTH, H. E. 1980. Defecation rates of captive brown bears, pp. 249-253, in C. J. Martinka and K. L. McArthur (eds.). Bears - Their Biology and Management, 4th Int. Conf. on Bear Research, 1977. Bear Biology Association.

SAHLÈN, V., SWENSON, J., BRUNBERG, S. and KINDBERG, J. 2007. Björnen i Sverige. En rapport från Skandinaviska Björnprojektet till den svenska Rovdjursutredningen. Skandinaviska Björnprojektet. Rapport 2006-4.

SCHALLER, G. B., HU, J., PAN, W., and ZHU, J. 1985. The Giant Pandas of Wolong. The University of Chicago Press, Chicago.

SCHULTE, B. A., MÜLLER-SCHWARZE, D., and SUN, L. 1995. Using anal gland secretion to determine the sex in beaver. *J. Wildl. Manage*. 59:614-618.

SLIWA, A. 1996. A functional analysis of scent-marking and mating behavior in the aardwolf, *Proteles cristiatus* (Sparrman, 1783). PhD dissertation. University of Pretoria, South Africa.

STØEN, O. G., BELLEMAIN, E., SÆBØ, S., and SWENSON, J. E. 2005. Kinrelated spatial structure in brown bears, *Ursus arctos*. *Behav. Ecol. Soc*. 59:191-197.

SUN, L. and MÜLLER-SCHWARZE, D. 1998a. Anal gland secretion codes for family membership in the beaver. *Behav. Ecol. Soc*. 44:199-208.

SUN, L. and MÜLLER-SCHWARZE, D. 1998b. Anal gland secretion codes for relatedness in the beaver, *Castor canadensis*. *Ethology* 104:917-927.

SWAISGOOD, R. R., LINDBURG, D., and ZHOU, X. 1999. Giant pandas discriminate individual differences in conspecific scent. *Anim. Behav*. 57:1045-1053.

TEGT, J. L. 2004. Coyote (*Canis latrans*) recognition of relatedness using odor cues in feces, urine, serum, and anal sac secretions. M.Sc. thesis, Utah State University.

TSCHANZ, B., MAYER-HOLZAPFEL, M., and BACHMANN, S. 1970. Das Informationsystem bei Braunbären. *Z. Tierpsychol*. 27:47-72.

WILSON, E. O. 1963. Pheromones. *Sci. Am*. 208:100-111.

WOLD, S., MARTENS, H., and WOLD, H. 1983. The multivariate calibration problem in chemistry solved by the PLS method, pp. 286-293, in B. Kågstrøm and A. Ruhe (eds.). Lecture Notes in Mathematics, Vol. 973. Springer-Verlag, Berlin.

WOOD, W. F., TERWILLINGER, M., and COPELAND, J. P. 2005. Volatile compounds from anal glands of the wolverine, *Gulo gulo*. *J. Chem. Ecol.* 12:2111-2117.

WYATT, T. D. 2003. Pheromones and Animal Behavior, Communication by Smell and Taste. Cambridge University Press, Cambridge.

YOGANAND, K., RICE, C. G., and JOHNSINGH, A. J. T. in press. Sloth bear *Melursus ursinus*, in A. J. T. Johnsingh and N. Manjrekar, (eds.). Mammals of South Asia. Universities Press, Hyderabad.

YUAN, H., LIU, D., SUN, L., WEI, R., ZHANG, G., and SUN, R. 2004. Anogenital gland secretions code for sex and age in the giant panda, *Ailuropoda malanoleuca*. *Can. J. Zool*. 82:1596-1604.

ZAR, J. H. 1998. Biostatistical Analysis. 4th ed. Prentice Hall, New Jersey.

ZEDROSSER, A., DAHLE, B., and SWENSON, J. E. 2006. Population density and food conditions determine adult female body size in brown bears. *J. Mammal*. 87:510-518.

ZHANG, J. X., DINGZHEN, L., SUN, L., WEI, R., ZHANG, G., WU, H., ZHANG, H., and ZHAO, C. 2008. Potential chemosignals in the anogenital gland secretion of giant pandas, *Ailuropoda melanoleuca*, associated with sex and individual identity. *J. Chem. Ecol.* 34:398-407.

ZHANG, J. X., NI, J., REN, X. J., SUN, L., ZHANG, Z. B., and WANG, Z. W. 2003. Possible coding for recognition of sexes, individuals and species in anal gland volatiles of *Mustela eversmanni* and *M. sibirica. Chem. Senses* 28:381-388.

ZHANG, J. X., RAO, X. P., SUN, L., ZHAO, C. H., and QIN, X. W. 2007. Putative chemical signals about sex, individuality and genetic background in the preputial gland and urine of the house mouse (*Mus musculus*). *Chem. Senses* 32:293-303.

ZHANG, J. X., SOINI, H. A., BRUCE, K. E., WIESLER, D., WOODLEY, S. K., BAUM, M. J., and NOVOTNY, M. V. 2005. Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. *Chem. Senses* 32:727-737.

ZHANG, J. X., SUN, L., ZHANG, Z. B., WANG, Z. W., CHEN, Y., and WANG, Y. 2002. Volatile compounds in anal gland of Siberian weasels (*Mustela sibirica*) and steppe polecats (*M. eversmanni*). *J. Chem. Ecol*. 28:1287-1297.

TABLE 1. RESULTS OF STUDIES OF CHEMICAL SEX DIFFERENCES IN GLANDULAR SECRETION IN MAMMALS

Species	Gland type[a]	Total no. of compounds	N ♂	N ♀	Significantly sex differences[b]								Reference
							Digital coding			Analog coding			
					No of compounds	GC	♂ specific compound	♀ specific compound	Cluster analysis	♂	♀	Color	
Giant Panda (*Ailuropoda melanoleuca*)	AG	95	11	13	-	-	0	0	No	5	4	-	Yuan et al., 2004
Steppe polecat (*Mustela eversmanni*)	AG	17[c]	11	10	-	-	0	0	-	4	3	-	Zhang et al., 2003
Siberian weasel (*Mustela sibirica*)	AG	14[c]	11	11	-	-	0	1[d]	-	1	4	-	Zhang et al., 2003
European otter (*Lutra lutra*)	AG	47	19	6	-	-	-	-	No	0	0	-	Bradshaw et al., 2001
Domestic dog (*Canis familiaris*)	AG	13	6	10	-	No[e]	0	0	-	-	-	-	Preti et al., 1976
Ferret (*Mustela furo*)	AG	>26[c,f]	3	3	-	-	0	1	-	4	3	-	Zhang et al., 2005

TABLE 1. CONTINUED

					Significantly sex differences[b]								
							Digital coding			Analog coding			
	Gland	Total no. of	*N*		No of		♂ specific	♀ specific	Cluster				
Species	type[a]	compounds	♂	♀	compounds	GC	compound	compound	analysis	♂	♀	Color	Reference
Eurasian beaver (*Castor fiber*)	AG	163	20	20	Yes	Yes[g]	7	1	-	-	-	Yes[h]	Rosell and Steifetten, 2004 Rosell and Sun, 1999
North American beaver (*Castor canadensis*)	AG	143	9	8	-	Yes[g]	-	-	-	-	-	-	Rosell and Steifetten, 2004
House mouse (*Mus musculus*)	PG	42	10	10	-	-	-	-	-	21	6	-	Zhang et al., 2007
Brown bear (*Ursus arctos*)	AG	90	4	7	No	Yes[g]	0	0	No	0	4	Yes	This study

[a] PG = preputial gland, AG = anal gland.
[b] - = not investigated.
[c] Investigated by headspace technique.
[d] Found in 7 of 11 females.
[e] Visually compared.
[f] 26 compound were selected.
[g] Partial least squares regression.
[h] Found in Rosell and Sun (1999).

TABLE 2. INDIVIDUAL NUMBER, SEX AND AGE OF BROWN BEAR (*URSUS ARCTOS*) DONORS OF FECES AND ANAL GLAND SECRETION

		Date of collection				Samples	
No.	Individual	Feces	AGS	Sex[a]	Age	Feces (*N* = 17)	AGS (N = 17)
1	W9101		25.04.2008	F	18		x[c]
2	W9301		18.04.2007	M	17		x
3	W0236		28.04.2008	F	15		x[c]
4	W0012	08.06.2007		M	15	x	
5	W0231	01.06.2007		F	15	x	
6	W0624	08.06.2007	21.04.2007	F	12-15[b]	x	x
7	W9403	08.06.2007	04.05.2007	F	14	x	x[d]
8	W0004	10.06.2007	24.05.2008	F	13	x	x[c]
9	W9903		30.04.2008	F	11		x[c]
10	W0424		30.04.2008	M	11		x[c]
11	W9806	08.06.2007		F	11	x	
12	W0717		18.05.2007	M	>10[b]		x
13	W0803		14.04.2008	M	>10[b]		x
14	W0626	08.06.2007	03.05.2007	F	8	x	x
15	W0716	08.06.2007		F	6-8[b]	x	
16	W0718	12.06.2007		M	5-7[b]	x	
17	W0209	08.06.2007		F	6	x	
18	W0217	05.06.2007	03.05.2007	F	6	x	x
19	W0517		20.04.2007	F	6		x
20	W0303	11.06.2007		F	5	x	
21	W0625	09.06.2007		M	4	x	
22	W0410	08.06.2007		F	4	x	
23	W0416		22.04.2007	F	4		x
24	W0415		20.04.2007	F	4		x
25	W0503	05.06.2007		F	3	x	
26	W0508		29.04.2008	F	3		x[c]
27	W0507	08.06.2007		F	3	x	
28	W0612		06.04.2008	M	3		x
29	W0619	08.06.2007		M	2	x	

[a] M = male, F = female.

[b] Exact age of these bears could not be determined the through age determination by the premolar tooth root.

[c] These bears were not used in analysis of total number of compounds, digital or analog coding.

[d] This bear was not used in the color comparison.

TABLE 3. COLOR OF THE ANAL GLAND SECRETION 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, P. O. Box 49022, S-100 28 Stockholm, Sweden.

TABLE 4. TENTATIVELY IDENTIFIED COMPOUNDS IN ANAL GLAND SECRETION OF BROWN BEAR (*URSUS ARCTOS*)

GC peak no.	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	No. of individuals Male (*N* = 4)	Female (*N* = 7)
1	11.243	Piperidinone	-		3
2	21.686	Tetradecene (hydrocarbon)	196		1
3	23.511	Pentadecene (hydrocarbone)	210		1
4	25.249	Hexadecene (hydrocarbone)	224		1
5	26.650	Hexadecanoic acid (palmitic acid)	256	2	1
6	26.960	Unidentified nitrogen compound	299		1
7	27.689	n-phenyl benzensulfonamide	233	1	
8	27.702	Unknown	-	2	2
9	28.191	Unknown	-	2	1
10	28.627	10,13-octadecadienoic acid, methyl ester	294	1	
11	28.733	Hydrocarbone c21	296	1	
12	28.818	Unknown	236		1
13	29.408	Octadecenoic acid (oleic acid)	282	2	1
14	29.749	Octadecanoic acid (stearic acid)	284	2	1
15	29.809	Hydrocarbone	-	1	3
16	30.219	Hydrocarbone C22	310	1	
17	30.376	Hydrocarbone	-	1	
18	30.699	Unknown	-	2	2
19	31.156	Unknown	226	1	
20	31.247	Unknown	-	2	1
21	31.286	Unknown	236	2	6
22	31.411	Unknown	-		1
23	31.642	Unsaturated wax ester C24	366	1	
24	31.796	Unknown	-	1	
25	32.540	Unknown	-	1	4
26	32.551	Unknown	-	1	
27	32.751	Unknown	250	4	7
28	32.813	Unknown	-	1	1
29	32.900	Unknown	-		2
30	32.929	Hexanedioic acid, dioctyl ester	370	1	
31	33.011	Hydrocarbone C24	338	1	
32	33.220	Unknown	-		2
33	33.324	Phenol, 2,2`-methylenebis(6-(1,1-dimethylethyl)-4-methyl)	340	1	
34	33.533	Unknown	-	1	
35	34.006	Unknown	-	1	
36	34.152	Unknown	264	3	7
37	34.301	Unknown	294	2	3
38	35.300	Unknown	324	2	
39	35.535	Unknown	462	1	3
40	36.750	Unknown	292	2	
41	37.295	Unknown	320	3	3
42	37.644	A steroid	368	3	2
43	38.362	Squalene	410	1	
44	38.531	Unknown	334	4	7
45	38.771	A steroid	368	3	2

TABLE 4. CONTINUED

GC peak no.	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	No. of individuals Male (N = 4)	Female (N = 7)
46	38.866	A steroid	366	1	
47	38.911	A steroid	366		1
48	39.144	A steroid	368	4	7
49	39.461	Hydrocarbone	364	3	4
50	39.527	Hydrocarbone	364	1	
51	39.749	A steroid	366	4	7
52	40.158	A steroid	-	2	2
53	40.555	Unknown	-	1	
54	40.655	Hydrocarbon	-		1
55	40.663	Unknown	-	2	2
56	40.837	Unknown	-	1	1
57	40.984	A steroid	380	4	7
58	41.808	Cholesterol	386	4	7
59	42.035	Unknown	394	2	4
60	42.177	A steroid	384	4	7
61	42.288	A steroid	386	3	6
62	42.323	A steroid	430	1	
63	42.409	Unknown	-	2	6
64	42.503	A steroid	414	2	2
65	42.519	A steroid	414	1	
66	42.660	Unknown	-		1
67	42.660	A steroid	-	1	
68	42.700	A steroid	382		1
69	42.735	Unknown	-	2	4
70	42.787	Ergost -5-en-o l (3 beta)	400	4	7
71	42.908	A steroid	412	3	2
72	43.029	A steroid	-	1	
73	43.066	A steroid	408	4	7
74	43.447	A steroid	428	4	7
75	43.610	Unknown	-	1	
76	43.689	A steroid	414	4	7
77	43.813	A steroid	-		1
78	43.868	A steroid	426	1	3
79	43.898	Lanosta-8,24-diene-3-o l (3-beta) (Lanosterol)	426	3	4
80	44.115	A steroid	422	4	7
81	44.423	A steroid	404	2	
82	44.486	Unknown	-		3
83	44.828	Unknown	430	2	1
84	44.831	Unknown	-	1	
85	45.124	Unknown	436	4	7
86	45.200	A steroid	-	2	
87	45.817	Unknown	476	1	
88	46.191	A steroid	450	1	
89	46.206	Unknown	-	1	
90	46.278	Unknown	450		4

[a] Mean value of the retention time.

TABLE 5. TENTATIVELY IDENTIFIED COMPOUNDS IN FECES OF BROWN BEAR

(*URSUS ARCTOS*)

GC peak no.	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	No. of individuals	
				Male (*N* = 4)	Female (*N* = 13)
1	1.382	Ethanol	-	4	13
2	1.504	Unknown	-		1
3	1.515	Methane, thiobis (dimetylsulfide)	62	1	1
4	1.539	Methylacetat	-	2	8
5	1.572	Sulfur compound	76	1	
6	1.622	Unknown	72	1	
7	1.629	Propan-1-o l	-	2	7
8	1.631	Unknown	-	1	5
9	1.755	Unknown	86	1	
10	1.800	Unknown	-	2	5
11	1.900	Unknown	82	1	1
12	1.901	Ethyl acetate	-	2	8
13	1.979	2-methylpropan- 1-o l	74		3
14	1.977	Unknown	-	1	
15	2.037	Acetic acid	-	1	4
16	2.194	Butanal, 3-methyl	86	3	7
17	2.283	Unidentified alcohol	-	2	6
18	2.280	Butan-1 o l	-	1	4
19	2.434	Unknown	-	1	
20	2.522	Propanoic acid, 2-methyl-,methyl ester	-		1
21	2.497	Unknown	101	1	
22	2.531	5 carbone ketone	-	1	4
23	2.558	Unknown	-	1	1
24	2.609	1,4.heptadiene	96	1	
25	2.661	N-heptane	-	1	
26	2.867	Etyhl propanoate	-	1	3
27	2.866	Unknown	-	1	1
28	2.904	Propyhl acetat	-	1	4
29	3.032	Methyl butanoate	-		3
30	3.239	Sulfur compound	94		1
31	3.235	Unknown	-	4	1
32	3.306	Unknown	-	4	9
33	3.418	Dimethyl disulfide	94	2	3
34	3.751	Sulfur compound	94		1
35	3.765	Propanoic acid, 2-methyl-,ethyl ester	-	2	2
36	3.894	Toluene	-	3	11
37	4.100	Unknown	-	2	2
38	4.184	Butanoic acid, 3-metyhl, methyl ester	-	1	4
39	4.182	Unknown	-		1
40	4.406	Butane 2,3-diol	90	1	
41	4.341	Unknown	-	1	
42	4.828	N-octane	-	1	1
43	4.828	Heptanal	114	2	2
44	4.936	Etyhl butyrate (ester)	-	2	4
45	5.911	Tetramethylcyclopentene	124		1
46	6.444	Butanoic acid, 2-metyhl, ethyl ester	-	2	3
47	6.538	Butanoic acid, 3-metyhl, ethyl ester	-	2	6
48	6.576	Unknown	-		1
49	6.604	2-ethylidene-1,1.imethyl, cyclopentane	124		1
50	6.623	Unknown	-	1	1

TABLE 5. CONTINUED

GC peak no.	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	No. of individuals	
				Male (N = 4)	Female (N = 13)
51	6.867	Unknown	119		1
52	6.959	Unknown	-	1	1
53	6.813	Unknown	-	1	1
54	6.962	Unknown	134		3
55	7.144	Isoamyl acetate	130	1	1
56	7.206	Unknown ester	130	1	
57	7.258	Santene	122		2
58	7.348	Unknown	-	3	9
59	7.439	2-heptanone	114	1	
60	7.443	Unknown	-		1
61	7.448	Unknown	-	1	
62	7.449	Nonene	126		1
63	7.396	Styrene	104		1
64	7.469	Pentanoic acid, 4-methyl, methyl ester	-		2
65	7.648	Unknown	-	2	5
66	8.026	Unknown	136	2	3
67	8.088	Unknown	-	3	8
68	8.239	Alpha-thujene	-	3	7
69	8.379	Alpha-pinene		4	13
70	8.655	Camphene	-	4	12
71	8.670	Unknown	-		1
72	8.744	Butanoic acid, 3-metyhl, propyl ester	-	1	2
73	8.780	Verbenene	-	3	9
74	8.954	Unknown	-	2	6
75	9.055	Unknown	-	1	2
76	9.059	Dimethyl trisulfide	126	1	1
77	9.109	M-Cymene	-	4	12
78	9.169	Unknown	-	1	1
79	9.225	Beta-pinene	-	4	13
80	9.306	Unknown	-		1
81	9.363	Unknown	138		1
82	9.400	Phenole	-	2	1
83	9.430	Phenole variant	-	2	6
84	9.510	Unknown	-	4	5
85	9.508	Unknown	-		5
86	9.674	N-decane	142	1	3
87	9.730	Unknown	-	1	2
88	9.735	Phellandrene	136		1
89	9.849	Delta 3 carene	-	4	13
90	9.958	Alpha-terpinene	136		1
91	10.042	Cymene isomer	134	3	8
92	10.086	Benzene, 1-metyhl-2(1-methylethyl)	-	4	11
93	10.162	Limonene	-	4	12
94	10.188	1,8,-cineole	-	1	2
95	10.322	Cymene isomer	134	1	2
96	10.400	Cymene isomer	134		3
97	10.536	Unknown	-	4	7
98	10.574	Unknown	142		1
99	10.647	Gamma-terpinene	136	1	3
100	10.655	Unidentified ester	-		1

TABLE 5. CONTINUED

GC peak no.	Retention time[a] (min)	Tentatively identified compounds	Molecular weight	No. of individuals	
				Male (N = 4)	Female (N = 13)
101	10.981	Unknown	-	2	7
102	11.000	Unknown	132		1
103	11.025	Unknown	150		1
104	11.026	Unknown	-		4
105	11.104	Terpinolene	136	1	4
106	11.124	Unknown	-		4
107	11.131	Undecene	154	1	
108	11.127	1-undecene	154	1	2
109	11.276	N-undecane	156	4	13
110	11.463	Unknown	-	2	4
111	11.792	Unknown	152		1
112	11.859	Pinocarveol	-	3	9
113	11.933	Unknown	-	2	9
114	11.935	Camphor	152	2	
115	12.031	5-methylundecane	170		1
116	12.143	Unknown	-		4
117	12.187	Pinocarvone	-	2	8
118	12.234	C12 hydrocarbone	170	1	1
119	12.233	Borneol	-	2	7
120	12.352	Isopinocamphon	-		3
121	12.422	Benzenemethanol, 4(1-ethylmethyl)	150		1
122	12.620	N-dodecane (hydrocarbone)	170	1	1
123	12.637	Myrtenol	-	2	7
124	12.811	Berbenone	-	3	5
125	13.092	Thymyl methyl ether	164		1
126	13.756	Bornylacetate	-	1	3
127	13.770	Tri-decene (hydrocarbone)	182	1	2
128	13.851	Unknown	-	3	1
129	13.855	Indole	117		1
130	14.135	2-hydroxy-5-methyl-methylbenzoate	166		1
131	14.572	Alpha-longipinene	204	1	2
132	14.826	Longicyclene	204		1
133	14.958	Beta bourbonene	204	1	2
134	15.150	Unknown	204		1
135	15.228	Isolongifolene	204	1	6
136	15.352	Beta caryophyllene	204		1
137	17.092	Unknown	-	3	7
138	17.320	Isopropyl dodecanoate	242		1

[a] Mean value of the retention time.

Figure legends

Figure 1

The study area in Dalarna and Gävleborg counties in Sweden.

Figure 2

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 glands, indicated by the wooden sticks (Foto: Andreas Zedrosser).

Figure 3

Gas chromatograms (GC) of the anal gland secretion from a typical female (a) and male (b) brown bear (*Ursus arctos*). Females seem to have a higher abundance in compound no. 57, 60 73 and 80, and males seem to have a higher abundance in compound no. 76. All these compounds are steroids. The numbers on the GC peaks correspond with compound numbers in Table 4. The *x* axis is the retention time in minutes and the *y* axis is the abundance.

Figure 4

Partial least square (PLS1) regression score plot showing the position of each gas chromatogram of brown bear (*Ursus arctos*) AGS (■ : Male (*N*=5);▽: Female (*N*=12)) of the two first components, PC1 and PC2. The numbers in the plots correspond with the number in Table 2.

Figure 5

Partial least square (PLS1) regression score plot showing the position of each gas chromatogram of brown bear (*Ursus arctos*) feces (■ : Male (*N*=4);▽ : Female (*N*=13)) of the

two first components, PC1 and PC2. The numbers in the plots correspond with the number in Table 2.

Figure 6

Dendrogram of hierarchical cluster analysis by using squared Euclidean distance for male and female brown bear (*Ursus arctos*) anal gland secretion (a) and feces (b). Labels indicate the bears sex and individual number, and “M” indicates male and “F” indicates female.

Figure 7

Sexual differences in relative abundance of compounds found in all males ▇ ($N = 4$) and female ▨ ($N = 7$), from the anal gland secretion of the brown bear (*Ursus arctos*). * $P < 0.1 > 0.05$ (marginal significance), ** $P < 0.05$, *** $P < 0.01$. The numbers on the x axis correspond with compound numbers in Table 4.

Figure 1

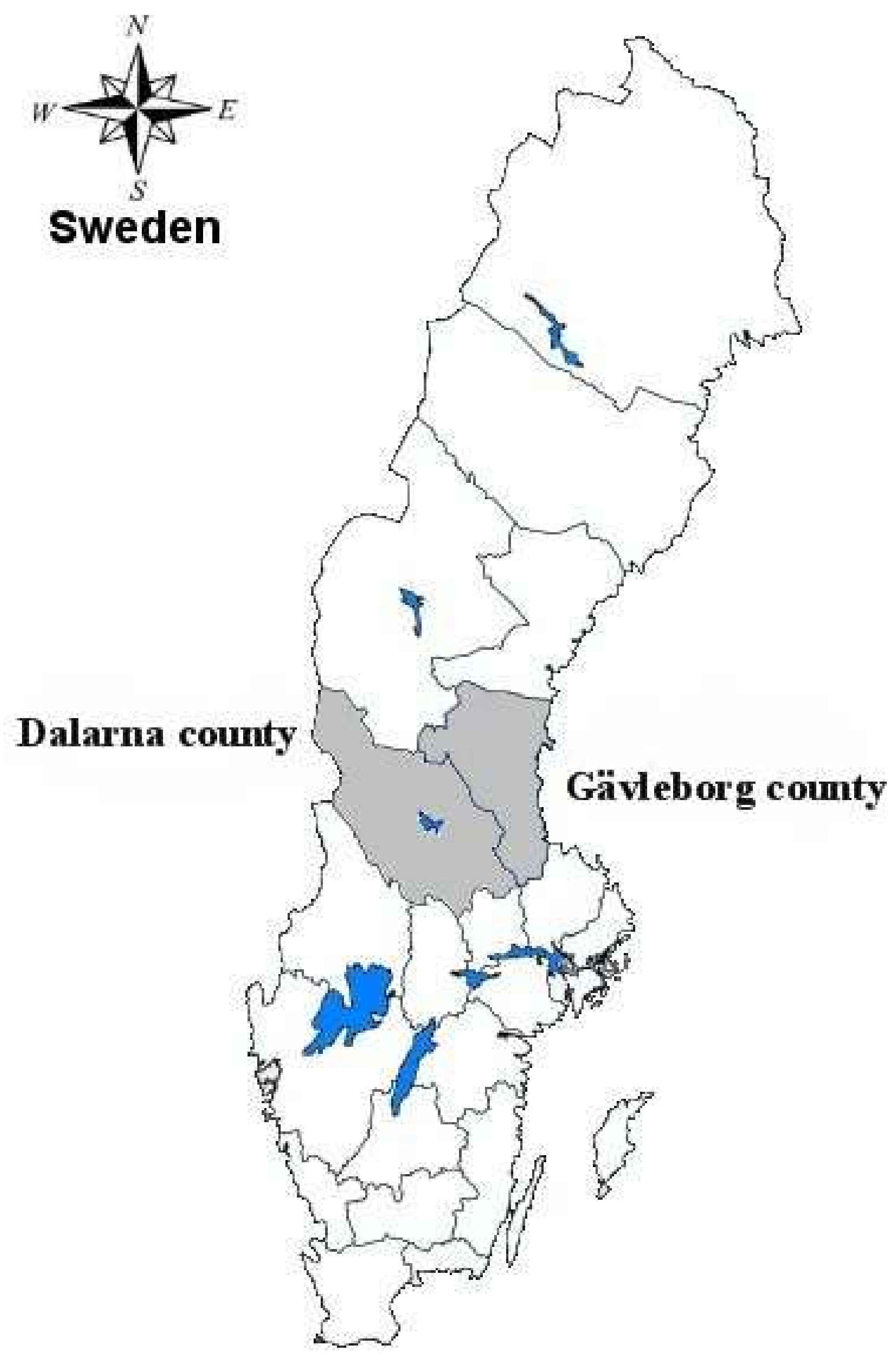


Figure 2

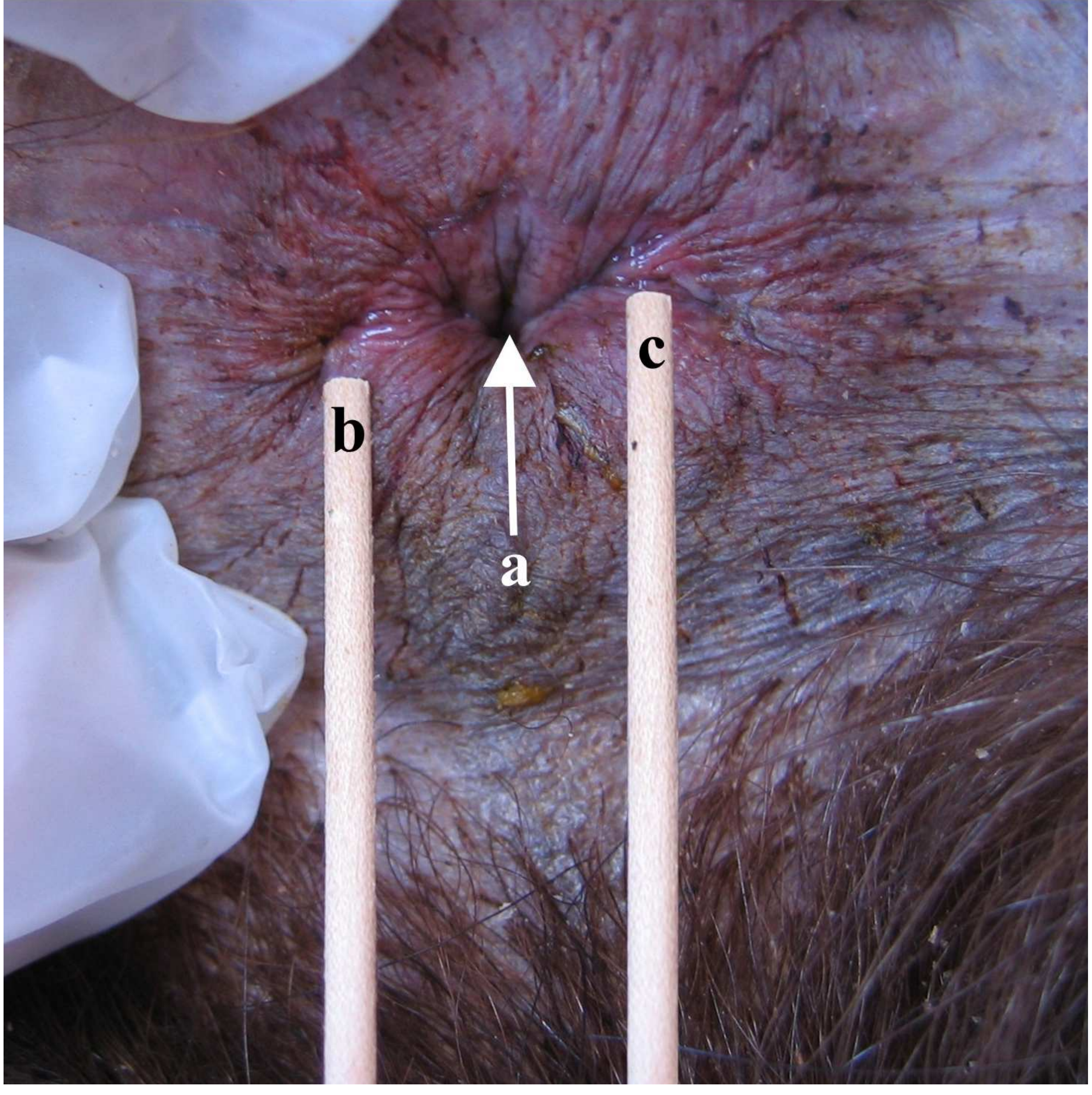


Figure 3

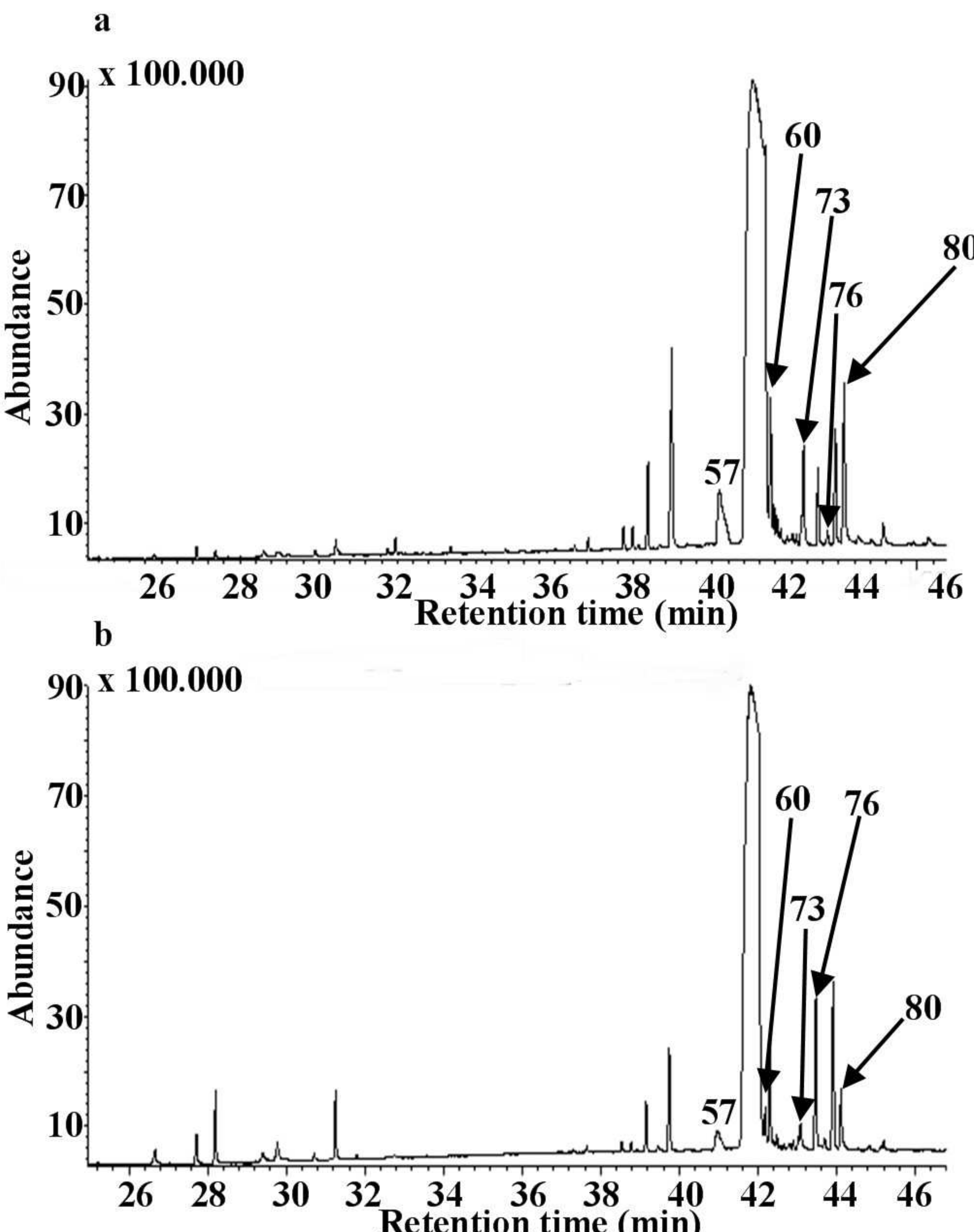


Figure 4

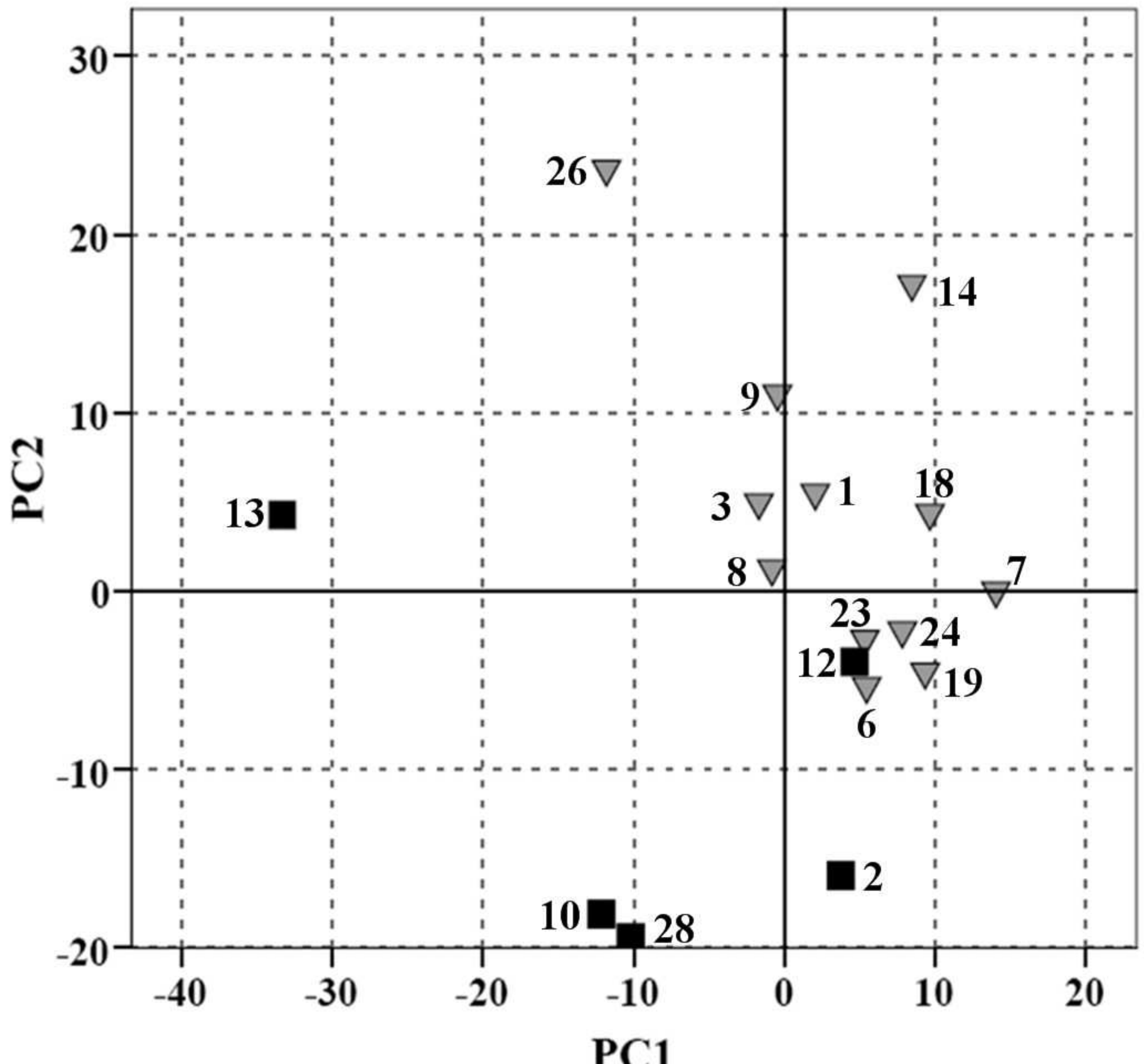


Figure 5

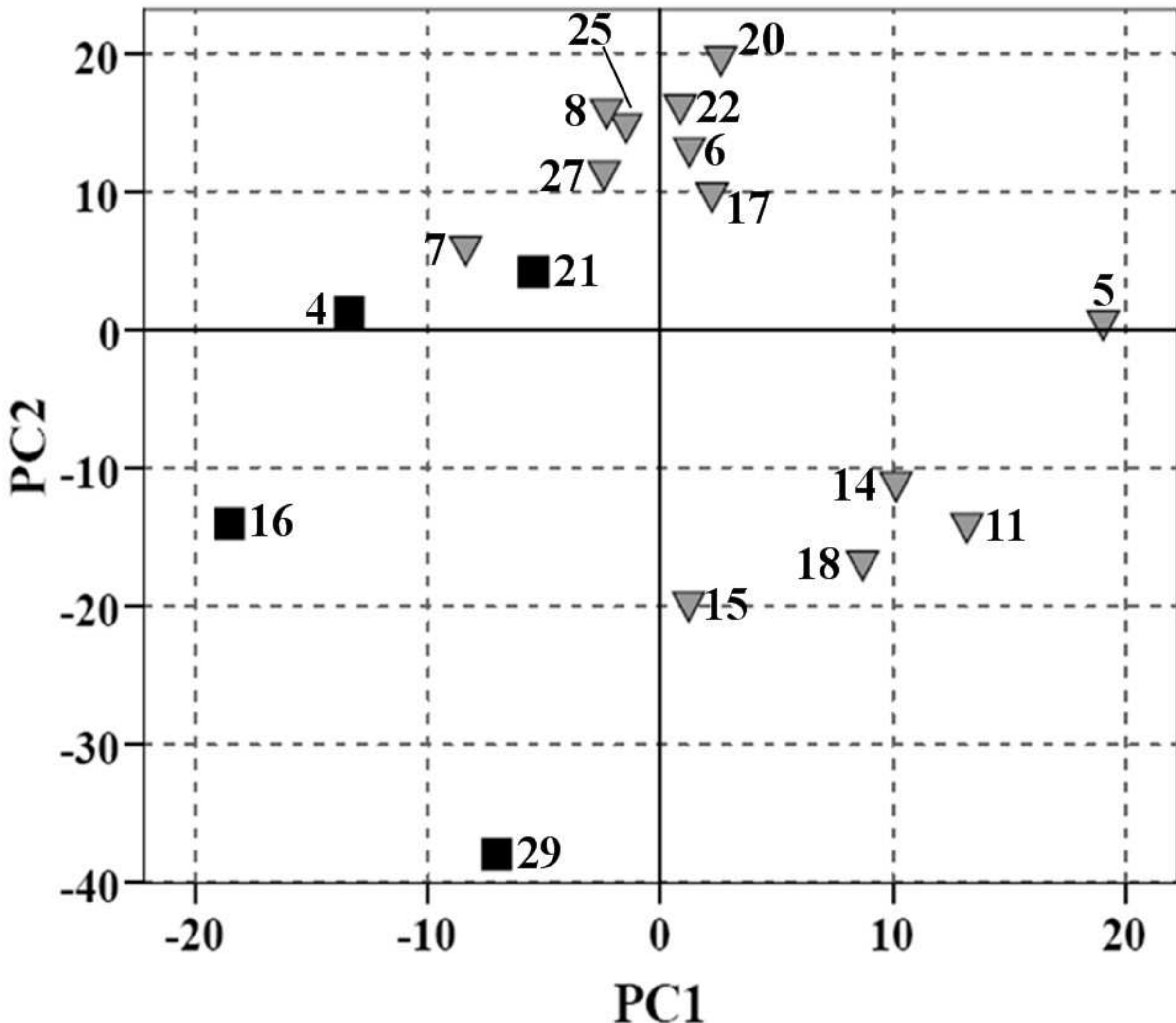
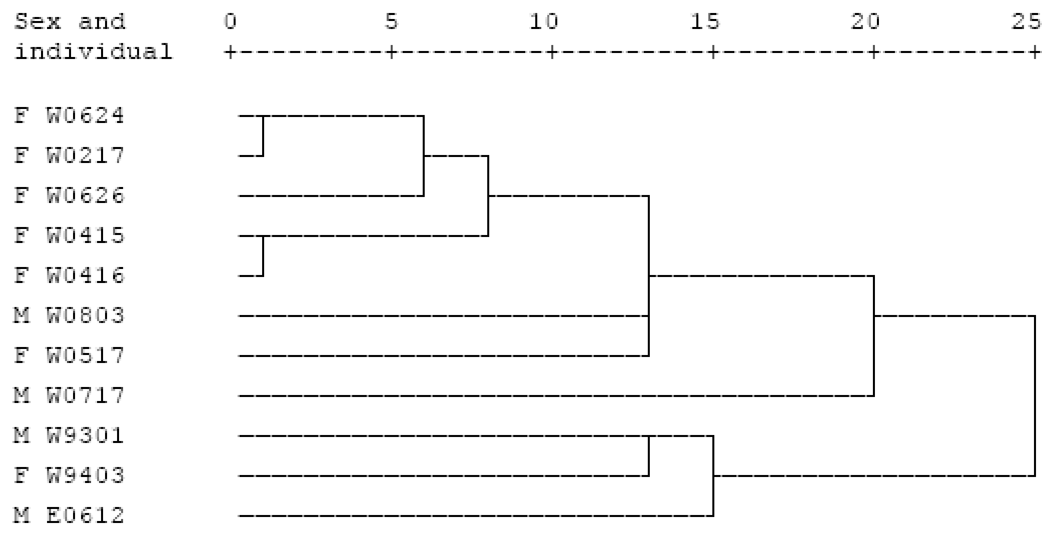


Figure 6

a



b

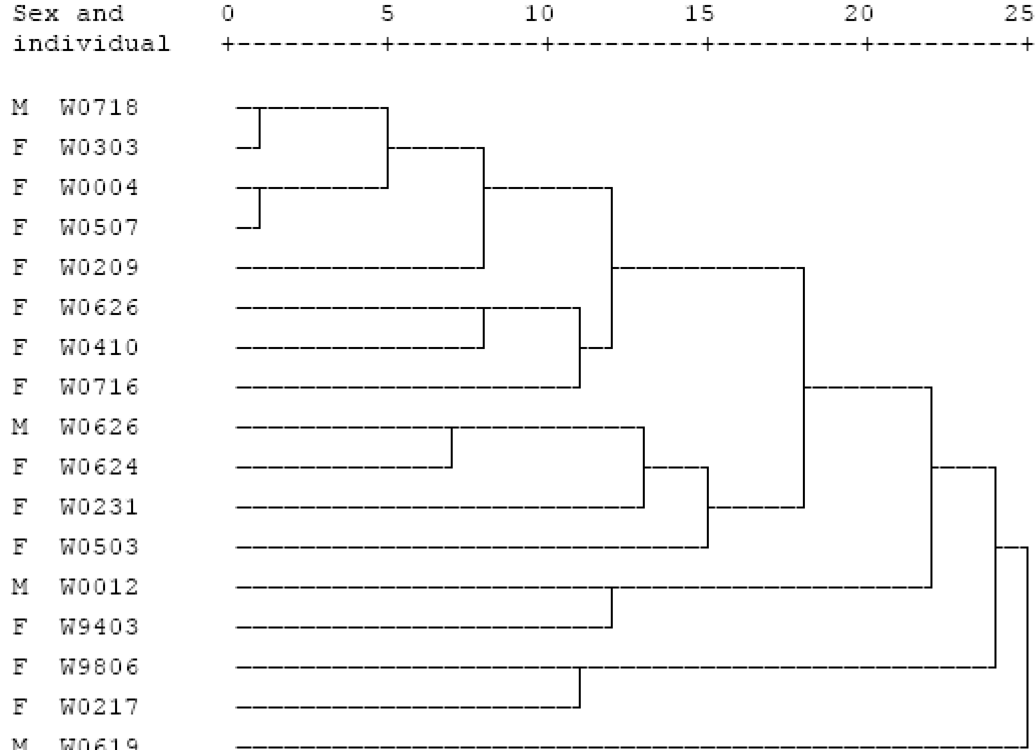


Figure 7