Brown bear skin-borne secretions display evidence of individuality and age-sex variation

Melanie Clapham
University of Victoria
Abbey E. Wilson
University of Saskatchewan
Candace L. Williams
Conservation Science and Wildlife Health, San Diego Zoo Wildlife Alliance
Agnieszka Sergiel (sergiel@iop.krakow.pl)
Polish Academy of Sciences

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Abstract

Scent originates from excretions and secretions, and its chemical complexity in mammals translates into a diverse mode of signalling. Identifying how information is encoded can help to establish the mechanisms of olfactory communication and explore the use of odours as chemical signals. Building upon existing behavioural and histological literature, we sought to examine the chemical profile of secretions used for scent marking by a solitary, non-territorial carnivore, the brown bear (*Ursus arctos*). We investigated the incidence, abundance, and uniqueness of volatile organic compounds (VOCs) from cutaneous glandular secretions of 12 wild brown bears, and assessed whether age-sex class, body site, and individual identity explained profile variation. The average number of compounds varied by age, but not solely by sex or body site. VOC profiles varied in composition and structure by age and individual identity (when individuals were grouped by sex), but not solely by sex or body site. Individual compound uniqueness varied by body site and age for both males and females and across individuals. Our results indicate that brown bear skin-borne secretions may facilitate age-sex class and individual recognition, which can contribute towards further understanding of mating systems and social behaviour.

Introduction

Chemical signalling facilitates both short and long-range communication in the majority of taxa. While social species frequently integrate concurrent modes of communication (visual, auditory, and chemical) [1,2], more solitary species tend to prioritise chemical signals as “bulletin boards" that broadcast long-lasting messages in the absence of the signaller [3,4]. Chemical signals are intended to influence the behaviour of conspecifics [5], usually to obtain or defend a resource and in sexual advertisement [6]. Signalling sexual receptivity and dominance should be equally important for solitary and social species to acquire fitness [7]. It has been suggested that the function of chemical signalling may be density dependent [8,9], and vary by the age, sex or social rank of the signaller [10,11]. The seemingly complex role of chemical communication in the social systems of solitary species is an ecological knowledge gap that requires further exploration.

Odour originates from excretions and secretions and its chemical complexity in mammals translates into a diverse mode of signalling [12–16]. Some species have evolved modified glands in particular areas of the skin, such as the face, flanks and appendages, which are selectively used to scent mark objects, self-anoint or allo-mark [4,15,17,18]. Glandular secretions from different body sites may convey different information by emitting different compounds and/or varying proportions of the same compounds [4,19–21], known as digital and analog coding of information, respectively [22]. Coding for sex and age within odour has been described in both digital and analog format [23–25] and individual chemical profiles can be discriminated by differences in relative abundance of compounds [26–28]. In solitary species, the ability to recognize individual odors can aid mate identification [29,30] and discrimination between familiar and unfamiliar conspecifics [26], with differences in associated risk. Identifying how information is coded can

not only facilitate understanding of the mechanisms behind chemical signalling in solitary mammals but also elucidate social function and broader ecological consequences.

Ursids are wide-ranging and largely non-territorial mammals who are believed to rely heavily on chemical signals to communicate with conspecifics. They are solitary but form aggregations at productive feeding sites, around oestrous females, and to play [31–34]. Evidence suggests that bears have evolved a highly developed olfactory system to process odours, enlarged or modified glands to deposit odour, and behavioural strategies to supplement the olfactory signal and reduce energetic costs [35–41]. Ursids deposit scent marks in the form of glandular secretions and urine onto the substrate and onto objects in their environment, mainly trees [37,38,42–45]. Specific postures have evolved in some ursids that are thought to deposit cutaneous (skin-borne) scent from different areas of the body, the most dominant being bipedal back (dorsal) rubbing, but also, flank and head rubbing [46–48] along with pedal marking [38]. Little investigation has been conducted into the use of urine and faeces as chemical signals for most ursids, despite observations of urination during pedal marking and rubbing [48,49]. Urine is used by some mammals for chemical communication, including giant pandas (*Ailuropoda melanoleuca*) [50,51] and wolves (*Canis simensis; C. lupus*) [52,53]. Giant pandas and brown bears (*Ursus arctos*) also possess anal glands, which giant pandas use extensively for chemical communication [27,54]. Less is known about the use of anal gland secretion (AGS) in other ursids, although Rosell *et al.* [25] found that brown bear AGS may code for sex and compounds were predominantly those of low volatility (molecular weight > 300); similar to giant panda AGS [27]. Chemical profiles from AGS and excreta may code for varying biological attributes compared to skin-borne secretions.

Recent histological investigations into sebaceous and apocrine glands in the dermis of brown bears dorsal region, found seasonal variation in their size and volume for male bears [40,41]. Other studies found male-specific compounds to be present in pedal secretion [38], but not anal gland secretion [25]. These studies form the only known chemical analyses of brown bear glandular secretions described in the literature. Behavioural evidence suggests that the function of chemical signalling in this species is to communicate dominance between males and/or attracting mates [9,35,55]. However, with still insufficient chemical evidence, signal content of cutaneous scent remains uncertain. Examining whether scent chemical composition varies would be the first step in determining if these signals code for different information according to the body region, or age and sex of the signaller, similar to Asian elephants (*Elephas maximus*) [11]. Chemical investigation in combination with existing behavioural evidence is therefore vital to facilitate the understanding of proximate and ultimate causes of this behaviour [56].

In this study, we analysed volatile organic compounds (VOCs) from cutaneous glandular secretions of wild brown bears to detect variation in chemical composition based on a) age-sex class, b) body site, and c) individual identity. We explored signal content by assessing VOC profile composition (digital coding) and structure (analog coding), as well as compound ‘uniqueness’ across a range of conditions (e.g., male and female within sex). We predicted that 1) chemical profiles would show age-dependent sexual dimorphism; 2) mature male chemical profiles would show more variation compared to mature females;

3) secretion from the dorsal region of the skin would show more variation than other body sites; and 4) chemical profiles would encode information on individual identity. By investigating the mechanisms of chemical signaling in a solitary, non-territorial carnivore, we can address knowledge gaps in aspects of ursid ecology such as social dynamics and mating behavior. In addition, results can inform the development of biomarkers for wildlife monitoring based on the association between chemical signatures and biological attributes (see [57,58]).

Methods

Site description

Sampling was conducted in southeastern Alaska, USA, across an area of 3,191km^2 along the Yakutat forelands (59°17'24"N, 138°53'14"W). The study site stretches from the Pacific Ocean to the west and extends 100 km east to border glaciers and mountains to the north and east. The majority of the site is within the United States Forest Service Tongass National Forest. Habitats range from intertidal flats, wetland shrubs and herbaceous vegetation, to Sitka spruce (*Picea sitchensis*) and western hemlock forests (*Tsuga heterophylla*). Food resources available to brown bears within the study area during late summer, include coastal strawberry (*Fragaria chiloensis*), small pelagic fishes (e.g., surf smelt (*Hypomesus pretiosus*)) and all five species of Pacific salmon (*Oncorhynchus* spp.). Density estimates for brown bears at the site are 98.8 ± 8.2 bears/1,000 km^2 [59].

Capture protocols

Brown bears were captured, immobilized, and fitted with GPS collars for a different study from July 2009 until September 2014 [see 59]. Capture and handling protocols were approved by Alaska Department of Fish & Game's Division of Wildlife Conservation Institutional Animal Care and Use Committee (protocol 2013-028) and were in line with procedures outlined by the American Society of Mammalogists [60]. Age of the bears captured was identified by extracting a premolar tooth and conducting cementum analysis [61]. For further details on capture methodology (including drugs administered and equipment used) see Crupi *et al.*[59].

Scent collection protocols

Samples for this study were collected from bears from 24 July to 23 September 2014. Twelve bears were captured in total during this sampling period, which included two mature males (15 and 19 years old), two mature females (14 and 19 years old), six young (independent subadults) males (3–4 years old) and two young females (4 and 6 years old; Table 1).

Table 1
Wild brown bears sampled in southeastern Alaska, USA.

Individual ID	Sex	Age	Age class[a]	Sample *n*
Y861	F	14	Mature	8
Y866	F	19	Mature	8
Y865	F	6	Young	4
Y867	F	4	Young	8
Y742	M	19	Mature	8
Y868	M	15	Mature	4
Y743	M	4	Young	4
Y744[b]	M	3	Young	2
Y860	M	4	Young	8
Y862	M	4	Young	8
Y863	M	3	Young	8
Y864	M	3	Young	8

[a] Young = < 10 years, Mature = ≥ 10 years

[b] This individual was excluded from the 'individual' treatment due to an incomplete dataset.

Samples were collected from four body sites: cheek (n = 20), flank (n = 20), hump (muscle between the shoulder blades; n = 19), and pedes (between the digits, plantar surface of the paw; n = 19). These body site sample collection locations were selected based on behavioural evidence of scent marking postures, and represent distinctive body parts most commonly rubbed against, or at the base of, marking trees [46]. Likewise, females and males of both young and mature age were sampled based on varying behaviour of these age-sex classes at marking sites, namely varying use of different scent marking postures [46,62] and frequency of marking [35].

To collect a scent sample, the hair was parted and medical-grade sterile cotton gauze was manually rubbed against the surface of the skin. The sample was then placed into 4 mL glass vials with Teflon PTFE-lined caps. This procedure was repeated per bear, sampling an adjacent patch of skin from the same body site and stored in a separate glass vial to collect a replicate sample. For sampling between the toes, the replicate sample was taken between the adjacent toes to the initial sample. Powderless nitrile gloves were worn at all times during scent collection. Gloves were changed or cleaned with Distel™ disinfectant wipes before sampling different body locations on each bear. Glass vials were frozen at -20°C as soon as possible after collection. Samples were shipped from Alaska, USA to British Columbia,

Canada for analysis in 2015. Samples were kept frozen during transportation using 4 Nu-Ice Marine Series Cooler packs (-16°C freezer charge) placed into a Yeti® Hopper™ cooler bag. Samples were then transferred to a laboratory freezer (-75°C) prior to analysis.

Chemical analyses

All samples (n = 78) were extracted and analyzed in 2017 by the British Columbia Ministry of Environment North Road Analytical Laboratory (Victoria, BC, Canada). Volatile compounds were extracted from sample gauze using 10 mL of methanol in a glass headspace vial (Perkin Elmer). Samples were hand-shaken for 5 seconds to ensure dispersion in the solvent. The vials containing the methanol and the sample gauze were introduced via an automated headspace sampler (Turbomatrix 110 Trap with helium carrier gas) into a gas chromatograph mass spectrometer (GCMS; Perkin Elmer Clarus 500, using a Zebron ZB-Waxplus Column (30m, 0.25mm ID, .25µm film thickness)). The headspace vial containing the extract was heated to 80°C and agitated for 15 minutes so that the analytes of interest migrated into and formed an equilibrium with the headspace in the vial. The headspace was sampled with the needle at 90°C and was allowed to desorb for 0.5 minutes. The headspace trap and purge as well as the GCMS settings were optimized using domestic dog (*C. familiaris*) hair to maximize sensitivity and accuracy of the instrument. Briefly, the GC oven was kept at 60°C for 1 minute, increased to 85°C at 3°C/minute, from 85 to 170°C at 8°C/minute, from 170 to 250°C at 20°C/minute and then held at 250°C for 8 minutes, resulting in a total run time of 31.96 minutes. The MS was operated in scan mode, using electron impact ionization and a scan range from 50–500 m/z.

Data processing

Initially, data files for each sample were converted to a common data format (.cdf files) using OpenChrom Software (Community Edition 1.3.0), which allows for the analysis and visualization of native data files from different mass spectrometry systems. One file failed to convert (pede sample from Y862) and was removed, resulting in n = 77 samples analysed. Next, files were converted to Agilent data files (.d files) using Agilent GCMS Translator software (Agilent Technologies, Santa Clara, CA) in order to utilize Agilent software workflows. We conducted deconvolution on chromatogram data in order to improve detectability, identification, and visualization of volatile organic compounds in samples using Agilent MassHunter Qualitative Analysis (for GC-MS) Workstation Software (signal to noise ratio threshold 0; absolute height 500 counts; absolute area 5000 counts) in conjunction with the Wiley Registry 10th Edition/NIST 2012 Mass Spectral Library [38,54,63]. Data files for each sample were then converted to compound exchange format (.cef files) and transferred to Agilent Mass Profiler Professional Software in order to align detected peaks across all samples [58,64]. Peak alignment allowed us to determine the presence and absence of compounds as well as the total number of compounds within and across samples. Several compounds were not identified in the library database with a reliable match factor (≥ 80); therefore, they are listed as mass@retention time (*sensu* [38]). Similar to previous studies, tentatively identified compounds were not confirmed with known standards, as most were not identified in the library database [25,27,65,66].

Data analysis

To establish whether biological attributes (sex, age, body site, and individual) explained the variation in VOC profiles, we assessed compound incidence (composition/digital coding) and abundance (structure/analog coding) using R statistical software (version 3.6.3) [67]. Replicate samples were included in analyses to ensure all potential volatile compounds were collected (as in [56,68]). This resulted in strict criteria for each compound to be included, as the relative abundance and presence of each compound had to meet certain thresholds in both samples, rather than using these metrics for one sample or an average across both samples. Compounds with an abundance of zero were replaced with half the value of the minimum abundance compound. The abundance of each compound was divided by the total abundance of all compounds in a sample to calculate the relative abundance of each compound to the overall scent profile [66]. We transformed the dataset using the square-root transformation to help achieve normality. To determine if data were homogeneous, we measured the variance across the different groups: 1) age class, 2) sex, 3) body site (nested by individual within sex), and 4) individual (nested in sex) (Supplementary Table S1). To evaluate how both VOC profile composition (incidence and abundance) and structure (incidence) varied initially by the aforementioned groups, and subsequently by 5) individuals nested within age-sex class and 6) individual and body site grouped by age-sex class, we used permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis and Jaccard distance matrices, respectively, with 10,000 permutations (*vegan*::adonis [69]), and only including compounds that occurred in at least 5% of all samples (4/77 samples).

Multivariate patterns were visualized using non-metric multidimensional scaling (nMDS) by generating distance matrices from the GCMS data (*vegan*::vegdist). Based on the goodness of fit and stress, three dimensions were used for the nMDS. Finally, compounds of interest were identified using similarity percentages (SIMPER; *vegan*::simper) when compounds contributed to the variation ≥ 2.0%. Each compound identified by the SIMPER analysis was analyzed using the previous model and evaluated as a univariate measure to determine compounds that varied with respect to mean relative abundance (*stat* package [70]). All data are expressed as the mean ± standard deviation (SD) and considered significant if $P < 0.05$, unless otherwise stated.

To explore compound uniqueness, we determined the presence and absence of compounds using Agilent Mass Profiler Professional Software and classified compounds according to their presence within and across conditions. Each of the nine treatments contained 2–11 conditions (e.g., treatment 'sex' = two conditions: 'female', 'male'), with samples sizes varying per condition (Table 2). Each condition contained samples from ≥ 2 individuals. We then created an 'individual' treatment where each individual bear was classed as a separate condition. VOCs were grouped into three categories (1) 'unique': present in 100% of samples within *only one* condition and absent in all other conditions, (2) 'signature dominant': present in 100% of samples in *at least one condition*, but may be present in fewer samples in other conditions, and (3) 'dominant': present in 100% of samples within *more than one* condition, and may be present in fewer samples within other conditions. To be included in analyses, compounds had to occur in 100% of

samples for at least one of the two (or more) conditions per treatment. Therefore, compounds could occur in more than one condition. For all treatments (except ‘individual’) this controlled for outlier compounds which could have been a result of individual variation in chemical profile or other biotic factors, but were not representative of a specific condition as a whole. Individual Y744 was not included in the ‘individual’ analysis due to an incomplete data set.

Table 2
Number of samples and conditions in each treatment comparison for analyses of compound uniqueness

Treatment	Sample *n*	Conditions	*n* per condition
Sex	77	2	28 (female) 49 (male)
Age class[a]	77	2	49 (young) 28 (mature)
Female; age class	28	2	12 (young) 16 (mature)
Male; age class	49	2	37 (young) 12 (mature)
Body site; female	28	4	7 (cheek) 7 (flank) 7 (hump) 7 (pedes)
Body site; male	49	4	13 (cheek) 13 (flank) 12 (hump) 11 (pedes)
Body site; female; age class	28	8	7 (cheek) 7 (flank) 7 (hump) 7 (pedes) 12 (young) 16 (mature)

[a] Young = < 10 years, Mature = ≥ 10 years

Treatment	Sample *n*	Conditions	*n* per condition
Body site; male; age class	49	8	13 (cheek)
			13 (flank)
			12 (hump)
			11 (pedes)
			37 (young)
			12 (mature)
Individual	75	11	8 (Y861)
			8 (Y866)
			4 (Y865)
			8 (Y867)
			8 (Y742)
			4 (Y868)
			4 (Y743)
			8 (Y860)
			7 (Y862)
			8 (Y863)
			8 (Y864)
[a] Young = < 10 years, Mature = ≥ 10 years			

Results

Using 77 wild brown bear samples (n = 12 individuals), we detected 254 compounds (5% incidence) across all samples. Representative total ion chromatograms are shown in Supplementary Figure S1.

Number of compounds

The average number of compounds varied by age (t(69.73)=-2.32, P = 0.023; Fig. 1), with samples from young bears having significantly more compounds than mature bears (young: 24.4 ± 9.2, mature: 20.1 ± 6.9; Fig. 1). However, no significant difference was found in the average number of compounds between females and males (females: 23.3 ± 6.6, males: 22.6 ± 9.7; t(72.72) = 0.42, P = 0.679; Fig. 1). Likewise, the average number of compounds did not vary by body site alone (cheek: 24.0 ± 9.4, flank: 23.2 ± 10.4, hump: 21.2 ± 8.1, pedes: 22.9 ± 6.4; F = 0.35, P = 0.787, one-way ANOVA).

Profile variation by compound incidence and abundance

Age, sex, and individual differences

Both the incidence and abundance of compounds varied significantly with respect to 1) age (Jaccard, P = 0.0014; Bray-Curtis, P = 0.0009) and 2) individuals nested within sex (Jaccard and Bray-Curtis, both P = 0.0001). These differences were visualized both overall (Supplementary Fig. S2) and separated by age class (Fig. 2). We found 23 compounds that contributed to the variation in the volatile profile (≥ 2%) of individuals nested within sex (Supplementary Table S2), and one compound (72.0@1.97) that contributed to the variation of volatile profiles within age class. Of these, we found the relative abundance of only three compounds to be significant across sex with respect to mean relative abundance (88.0@24.87, SIMPER 2.28%, P < 0.001; 2,5-Dichlorobenzyl alcohol SIMPER 2.09%, P < 0.001; 74.0@24.95 SIMPER 2.93%, P = 0.022).

Within age-sex classes, we found significant differences in compound incidence and abundance between individual bears with the exception of mature males (Supplementary Table S3). The differences in variance observed can be illustrated by nMDS, with young males and mature females having larger sample variance and little overlap compared to mature males with smaller variance and a strong overlap of ellipses (Fig. 2). We found 12 compounds that contributed to the variation in the volatile profile within mature bears and 15 compounds that contributed to the variation within young bears (separated by individuals nested within sex) (Supplementary Table S4). For example, 88.0@26.37 was found in higher relative abundance in mature male bears compared to their female counterparts, or young bears, with male Y742 displaying the highest relative abundance (Fig. 2c).

Body site differences

We did not find the incidence or abundance of compounds in the volatile profiles to vary across body sites nested within individuals (Jaccard, P = 0.41, Bray-Curtis, P = 0.38; Supplementary Table 3). Since significant differences were detected by age, and individuals nested within sex, samples were then grouped by age-sex class to determine if differences in both incidence and abundance were observed across body sites within individuals. Body site was not found to be significantly different for either measure across all classes (Bray-Curtis and Jaccard, both P > 0.05; Fig. 3).

Compound uniqueness by condition

We found unique (100% of samples within *only one* condition and absent in all other conditions), signature dominant (100% of samples in *at least* one condition, lower % in others), and dominant (100% of samples in *more than one* condition) compounds varied by (1) body site and age in females and (2) body site and age in males. We did not find unique, signature dominant, or dominant compounds to any condition in comparisons between sex only, age only, females by age, and males by age.

Body site and age variation in females

Within mature and young female bears (n = 4 individuals): one unique, 21 signature dominant, and 5 dominant compounds were detected in samples across body sites (Supplementary Table S5). Flank secretions collected from young females had the greatest number of signature dominant compounds (11), while cheek secretions collected from young females contained the lowest number of signature dominant compounds (1). One signature dominant compound (72.0@26.59) detected in samples collected from the flank of mature females only occurred in body sites from mature females, while two signature compounds (62.0@2.63 and 92.0@4.53) detected in samples collected from the flank of young females were only found in body sites from young females. Two compounds, 88.0@23.15 and 88.0@22.72 were found across all samples collected from the flank and interdigital secretions of mature females and the cheek secretions of young females. The only condition not to have a signature dominant compound was the hump of mature, female bears. One unique compound (62.0@2.63) was found in secretions collected from the flank of young female bears; this compound did not occur in any other conditions.

Body site and age variation in males

Within mature and young male bears (n = 8 individuals): zero unique, 17 signature dominant, and four dominant compounds were detected in samples collected across body sites (Supplementary Table S6). No signature dominant or dominant compounds were found in the secretions of young male bears from any body site, which could be an effect of a higher sample size for this age-sex class, compared to other age-sex classes. Hump samples collected from mature males had the greatest number of signature dominant compounds (9), while cheek and interdigital gland secretions had the lowest (2). Four dominant compounds occurred in mature male samples compared to young males. For example, 72.0@1.96 and 74.0@24.94 were detected across all samples collected from the cheek and flank of mature males, but were only present to a lesser extent in young males.

Individuals

Zero unique, 10 signature dominant, and one dominant compound were found across individuals (Supplementary Table S7). Ten signature dominant compounds were found in five of 11 bears: two mature males (Y742; Y868), two young males (Y743; Y860) and one young female (Y865); however, this varied for each individual. For example, four signature dominant compounds (86.0@25.01, 59.0@1.79, 74.0@25.24, and 74.0@25.37) were detected in samples collected from mature male Y868, while only one signature dominant compound (74.0@24.94) was detected in samples collected from mature male Y742. One dominant compound (72.0@1.96) was found in all samples collected from young female Y865 and mature male Y868. No dominant or signature dominant compounds were found for two mature females (Y861; Y866), one young female (Y867) and three young males (Y862; Y863; Y864).

Discussion

Odors play a key role in mammalian communication, conveying information about identity, sex, social status, reproductive state, group membership and/or territorial boundaries [12,15,71]. We sought to

understand mechanisms of olfactory communication in brown bears by investigating the chemical components of skin-borne secretions used in scent marking. Although our sample size is relatively small (n = 12 individuals), to our knowledge we are the first to examine the chemical composition of brown bear cutaneous secretions beyond pedal scents and anticipate this as a starting point for further inquiry.

Chemical profiles code for age

We found strong evidence of age-dependent variation in VOC profiles of coastal brown bears. Age class was a significant predictor of the average number of compounds and chemical profiles varied according to age in composition and structure. Contrary to our prediction on age-dependent sexual dimorphism, age did not require grouping with sex to be a significant predictor of compound variation. Age-related differences in chemical profiles have also been found in giant pandas [27], red deer (*Cervus elaphus*) [72,73], Eurasian otters (*Lutra lutra*) [24,74], mandrills (*Mandrillus sphinx*) [56], and white rhinos (*Ceratotherium simum*) [75]. In addition, behavioural indications of age signals in excretions and secretions have also been found in black rhinos (*Diceros bicornis*) [76] and giant pandas [77].

Age-related differences in chemical profiles have been shown to be hormonally regulated in mammals [78], which with physiological maturity, affect the chemical constituents of secreted scent [24,56]. In this study, skin-borne secretions of young bears had a higher number of compounds than mature bears, and one specific compound contributed significantly to age-related differences in chemical profiles. Investigations into the influence of symbiotic microbes on mammalian odours have found similar variation in bacterial communities by age class. Endocrine signalling and microbiota have a bi-directional relationship, where hormones can drive shifts in microbial composition or microbes can transform hormones as well, thus altering VOC composition (reviewed by [79,80]). This dynamic relationship can lead to differences in microbial composition related to age and reproductive cycle, thus driving changes in chemical composition associated with each (reviewed by [81]). This phenomenon has been observed in striped hyenas (*Hyaena hyaena*), where scent pouch composition and structure varies by age-class, with adults possessing a core suite of microbiota and younger animals showing more variation [82]. Similarly, the microbiota within anal glands of meerkats (*Suricata suricatta*) varied between males and females only after individuals were sexually mature, suggesting that physiological maturity was a catalyst for these changes [83]. Age determination from chemical signals could have fitness advantages for both young and mature bears. With some variation, brown bears reach sexual maturity at ~ 5 years of age for both males and females [84]. However, older male bears have higher reproductive success [85], females select larger males, and age is highly correlated with body size for males [86]. Likewise, females between nine and twenty years old produce the most offspring [87]. Age determination could function in mate selection and competitor assessment for mature bears, and may reduce risk to younger bears through honest signalling (*sensu* [88]). For example, in Asian elephants, the musth of young males varies from mature males in composition, and is thought to convey a non-threatening chemical message of naivety to avoid conflict with older males [11]. A signal of subordinance (low competitive ability) would also be advantageous for

brown bears, as a solitary carnivore that exhibits breeding and foraging aggregations, and female matrilineal assemblages [89].

Brown bears in this study were divided into age classes based on the demographics of the dataset; the difference between the eldest bear in the class 'young' and the youngest in the class 'mature' was eight years. This ensured a representative division according to life stage, and we believe reduced error in assignment concerning young adult bears (5–6 years of age). Comparative studies should be aware of the potential for varying results based on age classification and between age groups in the analysis of age-related chemical signals (*sensu* [82]).

Chemical profiles code for individuality within sex

Chemical signals of individuality are well documented in terrestrial mammals, including mustelids [66,90], rodents [91–93], primates [56,94] and carnivores [28,63,95]. In line with these studies, our results suggest that individual identity is coded in both the composition (digital coding) and structure (analog coding) of brown bear chemical profiles. While we found no unique compounds between individual bears, our prediction on profiles coding individual identity was supported: compound incidence and abundance varied by individuals within sex and when individuals were grouped by age-sex class. Individual compound uniqueness also varied according to individual bears. Mammalian chemical profiles are known to be complex mixtures [96] of a variety of chemical components that vary in incidence and abundance, resulting in distinctive odours [28]. Adding additional complexity, we found that compound uniqueness showed interindividual variation, with some compounds expressed in all samples from one individual, but absent from another. However, we did not find consistent compound uniqueness specific to each individual in the study.

The ability to discriminate individuals based on their chemical profile would provide considerable benefits for species that are able to retain this information for later use, and modify their behaviour in such a way that reduces risk and/or provides a benefit [92]. The presence of individual scent signatures does not necessitate their use in individual recognition, nor that they have evolved for this function. Nonetheless, if individual recognition contributes to fitness, individually distinctive odours should be used by other animals to gain information on conspecifics and their use as a chemical signal would evolve. Individuals that advertised their distinctive odours (e.g., through scent marking) would then have a competitive or reproductive advantage, especially if their odour also contains coded information on age and sex. Bears are thought to possess the ability to recognise previous mates [97], kin [89,98], and other conspecifics [99,100], and scent marking has been proposed as a method of signalling dominance and mate attraction [9,35]. Indeed, Morehouse *et al.* [101] recently found a positive relationship between tree rubbing and reproductive success for both male and female brown bears, and Hansen *et al.* [100] found that familiarity between female bears was important for home range settlement, which they suggest is facilitated in part by scent cues. Although brown bears are not a gregarious species, they do have some ecological traits (e.g., breeding and feeding aggregations, overlapping home ranges) that lead to conspecific interactions and

result in a social hierarchy. Chemical signals that encode individuality could facilitate individual recognition within these social contexts.

Our finding that profile composition and structure varied according to individuals nested in sex, also indicates that some of the variability may be due to differences between sexes. We found no sex-specific differences in average number of compounds, but compound uniqueness varied by both body site and age when grouped by sex. Contrary to our prediction on mature male profile variance, mature male bears were the only age-sex class where composition and structure did not vary significantly between individuals, and mature male individual profiles showed less variance than mature females and other age-sex classes. Mature male brown bears are considered to be the dominant age-sex class based on behavioural observations [102,103], and have been shown to engage more in scent marking via tree rubbing than other age-sex classes, including frequency and time investment [45,46]. As chemical profiles in brown bears appear to encode a signal for age (see above), which for other species conveys an honest signal of competitive ability [11], perhaps a mature male chemical signature - a signal of high competitive ability - is also present here. We are unable to develop this hypothesis further due to low sample size of mature males in our study (n = 2 individuals, 12 samples), however behavioural evidence indicates adult male-to-male signalling via scent marking in some populations [35,45] and evidence from spotted hyena (*Crocuta crocuta*) indicates that the scent profile of high-ranking females contains an 'olfactory badge of status' [104]. Further work is needed to test this hypothesis on a larger sample size of adult male brown bears.

Body site variation and compound uniqueness

We found no body site-specific differences in the average number of compounds, and no significant variation in profile composition and structure between body sites, or when body sites were tested within individuals and grouped by age-sex class.

Our results indicated that compound uniqueness varied by body site and age within sex, and samples from the dorsal (hump) region of mature males showed the highest level of compound uniqueness across males; giving partial support to our prediction of increased variation of hump samples compared to those taken from other body sites. In behavioural studies, dorsal (back) rubbing is a core marking posture, particularly for adult males [46,105,106]. Histological and histochemical analyses of the hump area of male bears have shown that sebaceous glands are enlarged and produce more oily secretion prior to and during the breeding season for intact males, influenced by testosterone concentration [41]. Similar was found for apocrine glands during the breeding season, indicating that both sebaceous and apocrine glands in the back of male bears vary by season and reproductive status [40]. As our samples were collected towards the end of- (July) and post-breeding season (August/September), chemical profiles may vary to those during the breeding season, especially in relation to body sites of male bears. Future studies should compare these results to samples taken during the breeding season, for both sexes. Histological and behavioural analyses in combination with the results presented here, provides a body of evidence that supports back rubbing as a focal method of chemical signalling for male brown bears.

It remains unclear why bears rub different body sites against trees (and other objects). Giant pandas are thought to mark urine using the handstand posture to deposit scent higher on trees, which communicates size and therefore competitive ability [3]. Our results indicate that while different body site secretions may contain different compounds, very rarely are they unique to that body site across either individuals or age-sex classes. In addition, the lack of variation across body sites within chemical profiles poses the question: why are brown bears using multiple body sites when marking with cutaneous secretions? One explanation that we propose is that these glands are relatively small, compared to highly specialised scent glands such as anal glands or sacs, and produce a smaller volume of secretion compared to urine or faeces excretion. Indeed, Alberts [107] proposed that by marking on elevated surfaces, signalling animals increase the active space of the scent mark and that for certain species, hair may facilitate the distribution of scent over a larger area. Therefore, marking with multiple body sites may increase the surface area of the overall scent mark, but not necessarily provide different coded information for brown bears, at least outside of the breeding season.

The surface may also influence the body site used, e.g., pedal marking the substrate [38] compared to, or in combination with, tree marking. We suggest further analyses of brown bear cutaneous scent focus on hump and pedal body sites only, to reduce potential redundancy. An exception to this could be examining the flank secretions of female bears (Results) [46] and the hump secretions of males during the breeding season.

We explored signal content and found that VOC profiles coded for age and individual identity, when individuals were grouped by sex. A suite of compounds varied in both composition (digital coding) and structure (analog coding) within chemical profiles, according to these attributes. Individual compounds were rarely unique to specific ages, sexes, individuals or body sites, instead we found their presence to vary by body site and age for both males and females. In combination with existing histological, chemical and behavioural analyses, our results indicate the presence of encoded chemical signals in cutaneous glandular secretions of brown bears. This study addresses an ecological knowledge gap for bears which can contribute towards further understanding of mating systems and social behaviour.

Declarations

Data availability

Data and code will be available prior to publication on GitHub at https://github.com/clw224.

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Author contributions

MC conceptualized the idea and facilitated data collection. MC and AS developed hypotheses. AEW led the processing of the analytical data, and CLW conducted data analyses and figure generation; together AEW and CLW interpreted results. All authors contributed to writing the manuscript.

Competing interests

The author(s) declare no competing interests.

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Figures

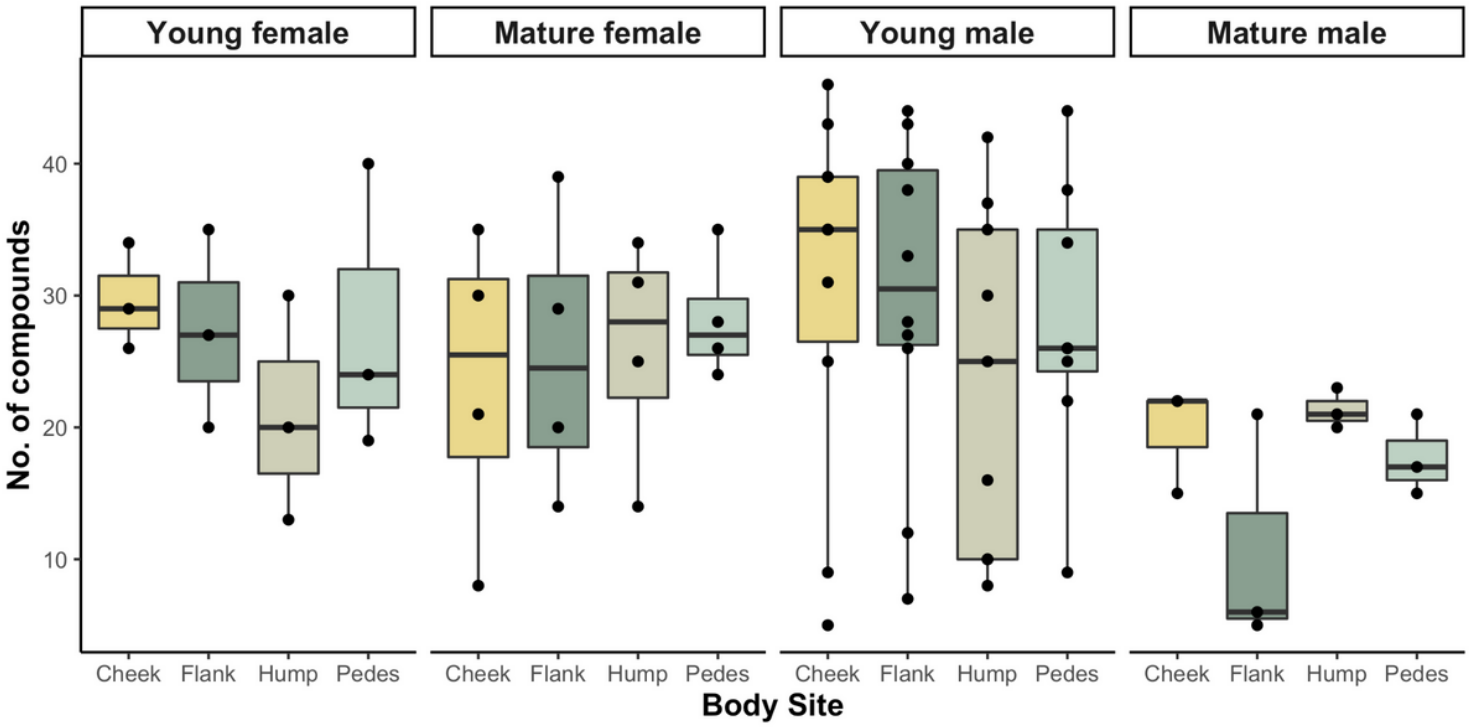

Figure 1

The mean number of compounds (>0.50 % relative abundance) in cutaneous scent samples, split by age-sex class and body site.

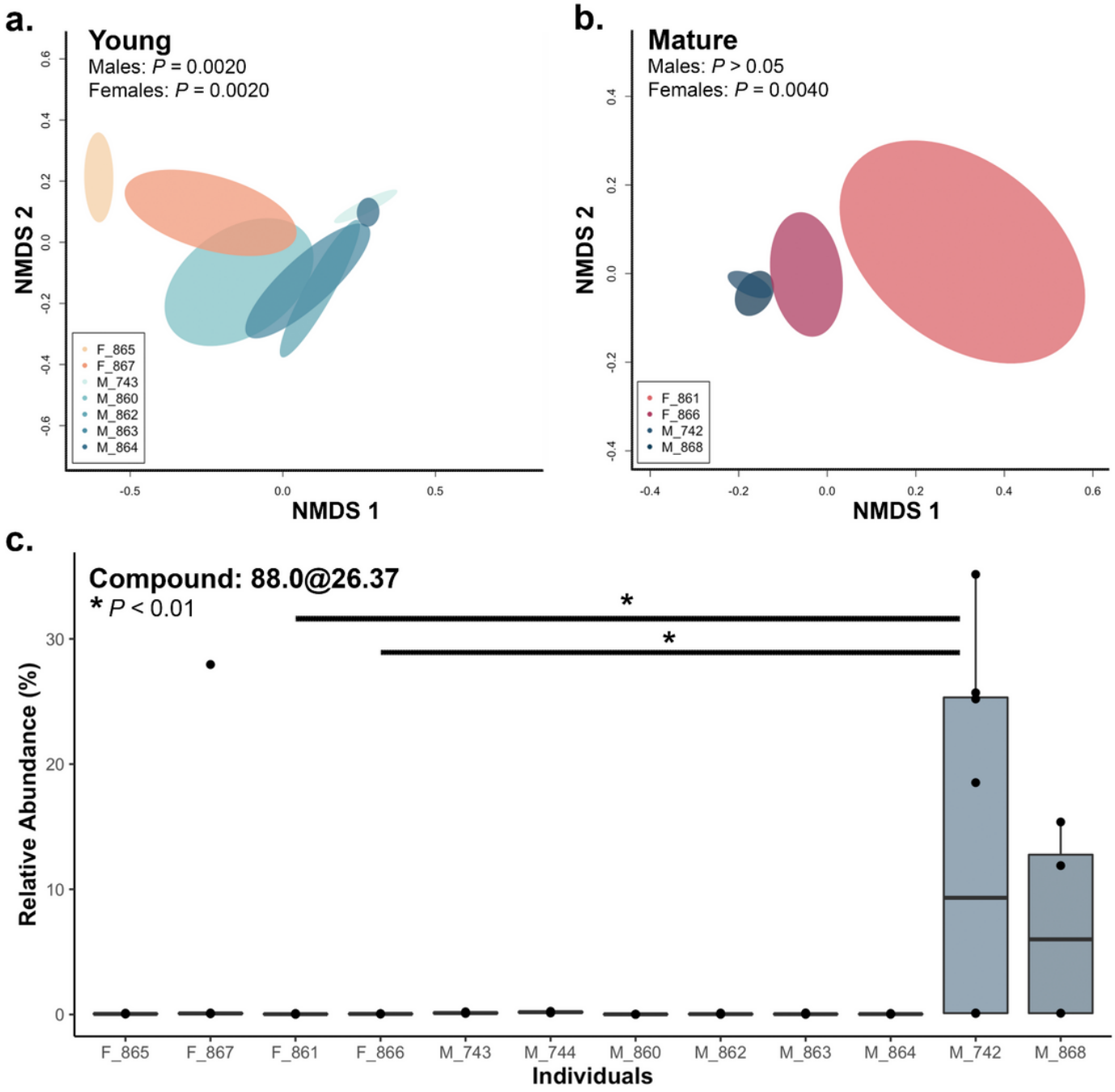

Figure 2

Nonmetric multidimensional scaling (nMDS, Bray-Curtis) analysis displays differences in volatile organic compounds in sample swabs collected from brown bears by age class for a) young bears (stress: 0.10, goodness-of-fit: 0.99) and b) mature bears (stress: 0.090, goodness-of-fit: 0.992), as observed by mass spectrometry. c) Relative abundance of compound 88@26.37 is found to drive variance between individuals. *Significant pairwise comparison with Bonferroni correction, both P<0.01.

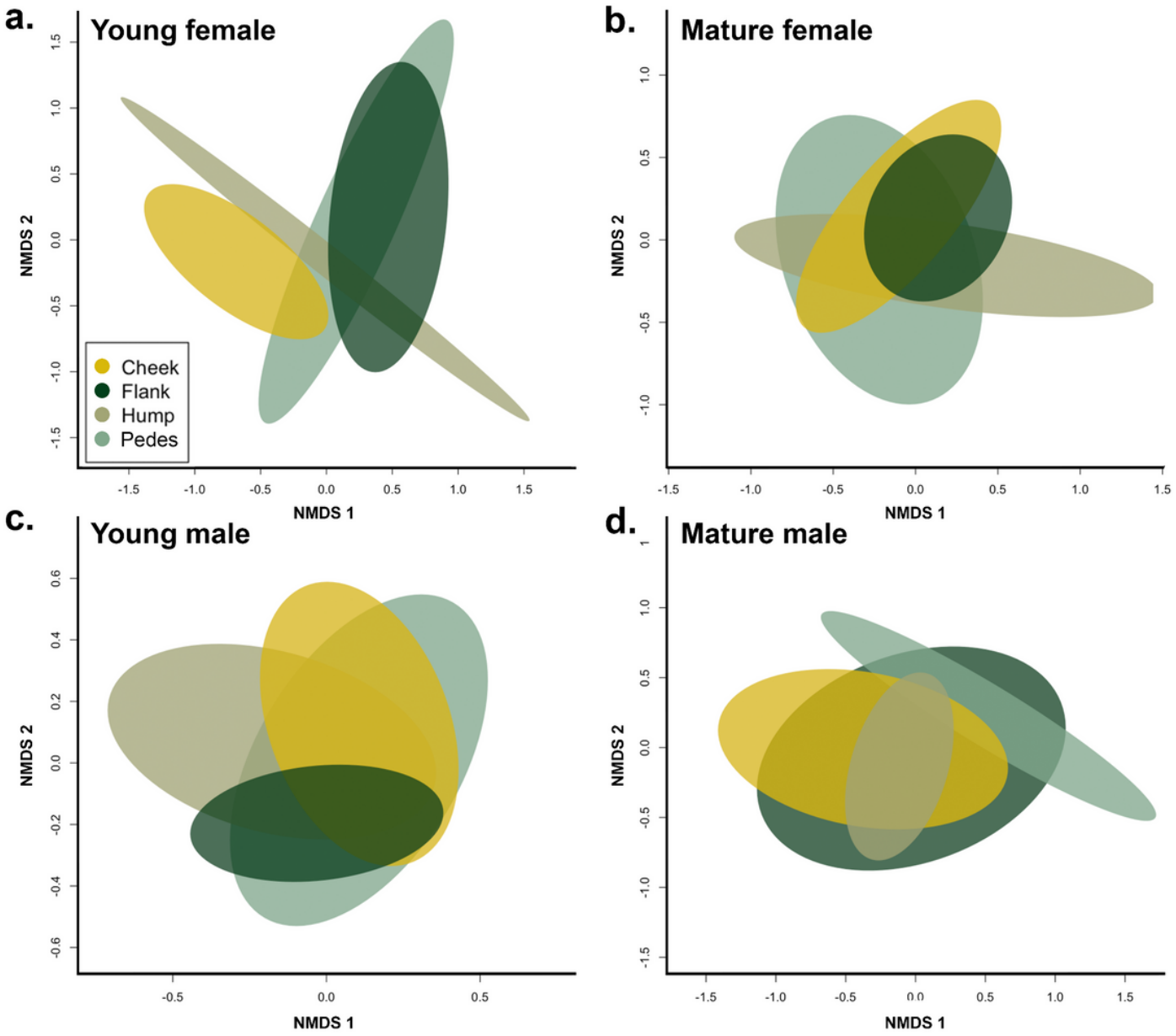

Figure 3

Nonmetric multidimensional scaling (nMDS, Bray-Curtis) displaying differences in body site within age-sex classes, a) young female (stress: 0.14, goodness-of-fit: 0.98), b) mature female (stress: 0.18, goodness-of-fit: 0.966), c) young male (stress: 0.23, goodness-of-fit: 0.945), and d) mature male (stress: 0.14, goodness-of-fit: 0.979) as observed by mass spectrometry.

Supplementary Files

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- SupplementaryInformation.docx