Anal scent gland secretions inform on sexual maturity, sex and social status in the Alpine marmot, *Marmota marmota* (Rodentia: Sciuridae): a role in intrasexual competition in cooperative breeders?

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In cooperative breeders, reproductive skew is extreme and leads to intense intrasexual competition for access to reproduction in both sexes. Given that securing the dominant position is costly, dominant animals should direct their aggressiveness towards same-sex subordinates that are potential competitors. Chemical communication has been reported to be involved in intrasexual competition in several mammalian species, and odour may be used as a cue to identify potential competitors. We predicted that odour should inform on sexual maturity, sex and social status in cooperative breeders. We tested these predictions in the Alpine marmot, a cooperatively breeding species, in which anal gland secretions are involved in aggressive behaviour. We sampled anal gland scents from 154 individuals of differing sexual maturity status, sex and social status. We found that anal gland secretions may inform on sexual maturity. When focusing on adult individuals, we found that anal gland scent differed according to sex and social status. Our results support the hypothesis that anal gland scent could be involved in intrasexual competition in Alpine marmots by allowing dominant individuals to target their aggressiveness towards same-sex competitors (i.e. sexually mature subordinates), resulting in the reproductive suppression of subordinates.

ADDITIONAL KEYWORDS: chemical label – dominance status – gas chromatography–mass spectrometry – odour cues – olfaction – sexual maturation – social species – wild population.

INTRODUCTION

Reproductive skew is extreme in singularly breeding species where a pair of dominant breeders is helped by non-reproducing subordinates (Silk, 2007). In cooperative breeders, this skew generates an intense intrasexual competition for access to dominance (and therefore to reproduction) in both sexes (Hauber & Lacey, 2005; Clutton-Brock *et al.*, 2006; Clutton-Brock & Huchard, 2013). Indeed, individuals that reach sexual maturity in those species may disperse in search of a dominant position in another family group or delay dispersal and stay in their family group as sexually mature but non-reproductive subordinates. Sexually mature subordinates that delay dispersal thus become potential competitors for the same-sex dominant, and this generates intrasexual competition within the family group. The risk of losing the dominant status compels dominant individuals to try to control the behaviour of subordinates to solve conflicts over dominance, and thus over reproduction (Cant & Johnstone, 2009; Clutton-Brock, 2009). Usually, they manage to secure their social status (and consequently their access to reproduction) either by expelling competitors from their social group (Dobson, 1982; Clutton-Brock *et al.*, 1998; Johnstone & Cant, 1999; Cant *et al.*, 2010) or by suppressing the

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reproduction of subordinates (Magrath *et al.*, 2004; Saltzman *et al.*, 2008; Young, 2009). Given that securing the dominant position is costly (e.g. by suppressing the reproduction of subordinates; Bell *et al.*, 2012), dominant individuals should target their aggressiveness towards potential same-sex competitors (Young, 2009; Clutton-Brock & Huchard, 2013). This requires dominant individuals to be able to recognize those competitors and subordinate individuals to be able to recognize the animals they have to challenge. Dominant and subordinate individuals can mutually identify themselves using different non-exclusive sources of information, such as visual (posture and behaviour), acoustic and olfactory cues (Candolin, 2003; Bretman *et al.*, 2011).

Chemical communication is widely used in the animal kingdom, and many studies have provided evidence for the importance of chemical communication in reproduction and social interactions (for review, see Johansson & Jones, 2007; Wyatt, 2014). Mammal scents provide information about signallers, such as species identity (e.g. delBarco-Trillo *et al.*, 2011,2012; Zabaras *et al.*, 2005), but also individual identity [scent may code for sex (e.g. MacDonald *et al.*, 2008; Kean *et al.*, 2011; Harris *et al.*, 2014), age (e.g. Buesching *et al.*, 2002; Kean *et al.*, 2011; Martín *et al.*, 2014) or sexual maturity (adult vs. subadult; e.g. Linklater *et al.*, 2013; Vaglio *et al.*, 2016; Spence-Aizenberg *et al.*, 2018)]. Several studies also reported that scent may inform on social status (e.g. Burgener *et al.*, 2009; Setchell *et al.*, 2010; Tinnesand *et al.*, 2013). Signalling of the social status of an individual has been interpreted in the context of intraspecific communication and territorial behaviour. Indeed, this information may be used in assessment of competitors by allowing conspecifics to evaluate the potential threat posed by an intruder (Gosling & Roberts, 2001; Tinnesand *et al.*, 2013). Chemical communication in relationship to intrasexual competition is expected to occur in cooperative breeders but is still poorly documented (Ziegler, 2013). If dominant individuals and subordinate competitors are able to recognize each other using chemical signals, we make the following predictions:

1. Odour should inform on sexual maturity. Therefore, we expect the odour of sexually mature individuals (adult animals) to be different from that of immature individuals.
2. Odour should inform on sex in adult individuals because competition for dominance (and reproduction) in singular breeders mainly occurs among same-sex individuals. Among adult individuals, we expect that odour should differ between males and females.
3. Odour should inform on social status in adult individuals. Therefore, we expect dominant individuals to have different odours from subordinate ones.

The Alpine marmot (*Marmota marmota*) is well suited to test these predictions. The Alpine marmot is a socially monogamous and cooperatively breeding species that lives in family groups of two to 20 individuals (Allainé, 2000). A family group is typically composed of a dominant pair, adult subordinates (sexually mature but non-reproducing) and immature (pups and yearlings) individuals of both sexes (Allainé *et al.*, 2000). Individuals of both sexes stay in their family group until they reach sexual maturity when 2 years old. Then, they can either disperse to gain dominance in another family group or delay dispersal and become potential competitors for the same-sex dominants in their family group (Allainé, 2000). Intrasexual competition for dominance occurs year-round and may result in the current dominant individual being evicted, evicting same-sex adult competitors or suppressing the reproduction of non-evicted subordinates (Arnold & Dittami, 1997; Hackländer *et al.*, 2003). This suppression is physiological and mediated mainly through aggression towards sexually mature subordinates (Hackländer *et al.*, 2003). Reproductive suppression is complete in subordinate females, whereas some subordinate males can successfully gain some extra-pair paternity (Goossens *et al.*, 1998; Cohas *et al.*, 2006).

Some observations suggest that chemical communication is involved in intrasexual competition in the Alpine marmot. Alpine marmots have three anal gland papillae that can be protruded from the anus, but their function is poorly known. However, this gland seems not to be used in territory scent marking (which is instead done by cheek rubbing with orbital glands; Bel *et al.*, 1995) but may still be used in intrasexual competition. Indeed, during encounters between adult members of a group, individuals often closely circle each other with flagging tails and then seem to expose their anal glands (D.A., pers. obs.), a behaviour also described during aggressive interactions between male yellow-bellied marmots (*Marmota flaviventris*) (Armitage, 2014).

Here, we investigate the chemical composition of anal gland secretions in a wild population of Alpine marmots, using gas chromatography–mass spectrometry (GC-MS). We aim to compare the chemical composition of individual marmot scents, which may differ according to sexual maturity status, sex and social status, and to identify which compounds are involved in these possible differences. We predict that the chemical composition of Alpine marmot anal gland scents is used in social recognition, particularly for intrasexual competition between same-sex individuals.

MATERIAL AND METHODS

Our study site was located in the nature reserve of La Grande Sassière (French Alps) at an elevation of 2400 m. Alpine marmots emerge from hibernation in early

April, and mating occurs during the 2 or 3 weeks after emergence (Psenner, 1957). Dominant females gestate for 30 days, and lactate pups in their natal burrows for 40 days; therefore, pups emerge between mid-June and mid-July (Psenner, 1960; Arnold, 1990). Between mid-May and mid-July 2016, we captured 154 Alpine marmots (detailed in Table 1) from 21 family groups using two-door live traps placed near the main burrows of each territory and baited with dandelions (*Taraxacum densleonis*). We tranquilized individuals using Zoletil 100 (0.1 mL kg^{-1}) and identified each animal with a transponder and a numbered ear-tag (placed on the right for the females and on the left for the males, for easier identification during field observations). Dominant individuals were also recognizable from a coloured plastic tag attached on the opposite ear. We estimated the social status of each adult (dominant vs. subordinate) based on scrotal development for males and development of teats for females, and we confirmed our assessment by behavioural observations (territorial scent markings; Bel *et al.*, 1995, 1999).

ETHICAL NOTE

The two-door live traps were checked every half hour to limit the time spent by a marmot in a trap and thus reduce its exposure to predators and the weather. Once captured, marmots were transferred in an opaque jute bag (to limit stress) to a field laboratory room where handling occurred. Once tranquilized, handling lasted a maximum of 10 min. The recovery did not require the use of an antidote, and the marmots were placed in a cool, quiet room for 15 min after handling to recover, until they were able to walk. All tranquilized marmots recovered well, and no adverse effects were noticed; all individuals were observed alive the day after their capture. Tranquilization of pregnant or lactating females did not have any obvious impact upon their offspring, because all the females successfully raised offspring to weaning. Overall, individuals were absent from their territory for a maximum of 40 min, and we never observed exclusion from the territory for any individual of any age after capture. Marmots were tranquilized no more than once a year and, in case of recapture the same year, the marmot was weighed and then released without additional handling.

All handling and sampling were done by T.Z. and Sylvia Pardonnet, who are authorized for experimentation with animals by the French Ministry of Agriculture and Fisheries (diploma no. R69UCBL-ENVL-F1-03 and no. R-13CNRS-F1-10). The protocol was approved by the University of Lyon 1 Ethical Committee (CEEA-55, protocol BH2012-92-V1), and the authorization to capture Alpine marmots was issued by the Préfecture de Savoie (arrêté préfectoral no. 2017/41) after approval by the advisory committee of the Nature Reserve of La Grande Sassière.

COLLECTION OF SCENT SAMPLES

After the Alpine marmots had been tranquilized, the anal glands were cleaned with ethanol to remove any environmental contaminants. Then, we collected odoriferous secretions by gently squeezing the area around the gland, wearing clean nitrile gloves to avoid contamination by human chemicals. Each sample was collected with a 50 μL glass capillary and placed into a 1.5 mL opaque chromatographic glass vial filled with 200 μL of dichloromethane solvent (HiPerSolv CHROMANORM for HPLC; VWR, Center Valley, PA, USA). In each 200 μL we added an internal standard, biphenyl (molecular weight, 154.21 g mol^{-1}, 99.5%; Sigma Aldrich, St Louis, MO, USA) at a concentration of 0.2 g L^{-1}. Vials were sealed with a Teflon-lined cap and stored at –20 °C in the field and at –80 °C in the laboratory until GC-MS analysis. In addition to these scent samples, several 'field control samples' (i.e. with solvent but without marmot secretions) were collected using the same protocol, to identify potential contaminants.

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS

Just before GC-MS analyses, we transferred all liquid scent samples (including 'field control samples') to 0.3 mL inserts in new, clean vials. We used an interfaced Hewlett-Packard 6890 gas chromatograph system equipped with a non-polar DB-5 MS column (30 m long × 0.25 mm internal diameter × 0.25 μm film thickness; Agilent Technologies) coupled with an HP 5973 MDS (mass selective detector) mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). We used helium as the carrier gas at a flow rate of 1 mL min^{-1} and an electron impact ionization of 70 eV. The temperature of injection was set to 300 °C, and 1 μL of sample was injected automatically in splitless mode after being vortexed to homogenize the scent sample. The oven temperature programme started with 4 min at 90 °C, then increased by 12 °C min^{-1} up to 210 °C,

Table 1. Number of animals sampled according to sexual maturity status, social status and sex

Sexual maturity status	Immature		Sexually mature			
Social status	Subordinate		Subordinate		Dominant	
Sex	Male	Female	Male	Female	Male	Female
Number of animals	57	34	22	11	14	16

Immature individuals are pups and yearlings. Sexually mature subordinates were 2 ($N = 28$) or 3 years old ($N = 4$), and dominant individuals were 3 years old ($N = 5$) or older, up to 12 years old ($N = 25$).

and then increased again by 5 °C min⁻¹ up to 310 °C, and finally was held at 310 °C for 5 min, resulting in a total programme time of 39 min. We ran blank samples containing only 200 µL of dichloromethane every seven samples. These controls provided an estimate of the potential noise related to the potential accumulation of some compounds along the column, for example.

IDENTIFICATION OF COMPOUNDS

We tentatively identified anal gland secretion compounds using the Kovats retention index (RI) and the mass spectrum obtained by Agilent MassHunter Qualitative Analysis software (B.07.01 version), which were then cross-checked with the National Institute of Standards and Technology (NIST) WebBook. We calculated RIs of compounds by means of a mixture of C_7–C_{40} alkanes with known RIs (Supelco; Sigma Aldrich, St Louis, MO, USA; 1000 µg mL⁻¹ diluted at 1:100 in hexane) run in identical gas chromatographic conditions. Whenever possible, we proposed the name of a compound or its chemical family.

CHEMICAL ANALYSES

Data acquisition and pretreatment

The area of every peak (i.e. integration of the area under the curve) was obtained by an automatic integration with Agilent MassHunter Qualitative Analysis software (B.07.01 version), and we manually checked each integration to avoid software errors. Then, we used AMDIS (automated mass spectral deconvolution and identification system) software to control the mass spectral similarity of peaks between all samples, in order to be sure of the peak identification from one individual to another. Furthermore, the internal standard (biphenyl) was used to control instrument drift over time. Three compounds were found in all field control samples and in all scent samples and were therefore considered as contaminants and removed from the analyses. We also removed compounds found in < 5% of the total individuals because they were considered to be too rare to contribute to the discrimination of each group (e.g. Harris *et al.*, 2012). Then, we calculated the relative abundance of each compound by converting each single peak area into a percentage of the sum of all compound areas for a given individual. Finally, we removed peaks with a relative abundance < 0.05% to exclude background noise (Drea *et al.*, 2013), and we square-root transformed the final data set to reduce the impact of the most abundant compounds upon our analyses (Clarke & Warwick, 2001).

Statistical analyses

We first tested the prediction that the odour of sexually mature individuals is different from that of immature individuals. We also tested whether odour informs on sex and differs between males and females. For that, we calculated Euclidean distances between every pair of samples to produce a resemblance matrix, from which we conducted a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001, 2017), using 9999 permutations on the full final data set to test whether chemical profiles differed according to sexual maturity status and sex.

As proposed by Drea *et al.* (2013), we carried out a principal components analysis on the correlation matrix to reduce the number of compounds and to highlight only those that explain most of the variance. We decided to retain compounds with a cumulative contribution on the first three axes > 50% of the total contribution. We then conducted a second PERMANOVA on the chemical data subset to check that this compound selection did not change results obtained with the first PERMANOVA. Finally, we performed a linear discriminant analysis (LDA) on the chemical data subset to investigate whether variation in the chemical composition of anal gland scents can be used to separate individuals according to their sexual maturity status and to their sex, and we specified the chemical compounds implicated in the previous analyses.

In a second step, we focused on sexually mature individuals, to test predictions that odour informs on sex and social status in adult individuals. We selected sexually mature individuals ($N = 63$) and again removed compounds found in < 5% of adult individuals. As before, we conducted a PERMANOVA using the same parameters, to test whether chemical profiles differed according to sex and social status. We used the same compound selection as before to conduct the second PERMANOVA, to verify that this selection would not change results. Finally, we performed an LDA on the chemical data subset, to investigate whether variation in the chemical composition of anal gland scents allows separation of sexually mature individuals by their sex and social status, and we again specified the chemical compounds implicated in the previous analyses.

All statistical analyses were conducted in R v. 3.4.3 (R Core Team, 2017), with 'ade4' (Dray & Dufour, 2007) and 'adegraphics' packages (Siberchicot *et al.*, 2017) for principal components analysis, the 'vegan' package (adonis2 function; Oksanen *et al.*, 2018) for PERMANOVAs and the 'MASS' package (lda function; Venables & Ripley, 2002) for the LDAs.

RESULTS

A total of 82 chemical compounds were detected in scent samples from 154 individuals (mean ± SD, 30.96 ± 5.17 compounds per individual). We found that the chemical composition of the anal gland secretion

of Alpine marmots contained a mixture of small volatile and large non-volatile chemical compounds, from piperidin-2-one (molecular weight, 99.13 g mol^{-1}) to lanosterol (molecular weight, 426.71 g mol^{-1}).

SCENT OF SEXUAL MATURITY STATUS

The PERMANOVA performed on the whole data set indicated a difference between sexually mature individuals and immature ones (pseudo-F $F_{1,150}$ = 80.68, P = 0.0001), but neither the sex effect (pseudo-F $F_{1,150}$ = 1.34, P = 0.22) nor the interaction between sex and sexual maturity status (pseudo-F $F_{1,150}$ = 1.15, P = 0.28) was significant. The principal components analysis led us to select 24 chemical compounds for the subsequent analyses (Table 2), which had a global contribution to the first three axes > 50%. The PERMANOVA performed on those 24 compounds gave similar results to the one performed on the whole data

set (sexual maturity status, pseudo-F $F_{1,150}$ = 117.10, P = 0.0001; sex, pseudo-F $F_{1,150}$ = 1.10, P = 0.30; and interaction, pseudo-F $F_{1,150}$ = 1.11, P = 0.29), indicating that no important chemical information was lost using our procedure of compound selection.

The LDA performed well at separating sexually mature from immature individuals on the basis of these 24 compounds (Fig. 1), correctly assigning 95.60% of sexually mature individuals and 96.83% of immature individuals. Five compounds characterized sexually mature individuals, whereas eight other compounds were strongly characteristic of immature individuals (Table 2). Specifically, the anal gland secretions of sexually mature individuals appeared to be characterized by a high relative abundance of various chemical families, such as alkaloid (piperidin-2-one), carboxylic acid (3-phenylpropanoic acid), lactone (5-dodecyloxolan-2-one) and steroids (cholestanol and cholest-5-en-3-one). In contrast, anal gland secretions of immature

Table 2. Chemical compounds retained in the linear discriminant analysis to separate individuals according to sexual maturity status

Number of compound	Retention time (min)	RI calculated	Molecular weight (g mol^{-1})	Name of compound proposed	Formula	Cos
1	7.075	–	99	Piperidin-2-one	C_5H_9NO	0.72*†
2	9.251	1328	150	3-Phenylpropanoic acid	$C_9H_{10}O_2$	0.68*†
3	11.9	1558	200	Dodecanoic acid	$C_{12}H_{24}O_2$	−0.52*‡
4	13.542	1720	226	Pentadecanal	$C_{15}H_{30}O$	0.43
5	13.72	1739	226	(Z)-9-Tetradecenoic acid	$C_{14}H_{26}O_2$	−0.44
6	13.886	1757	228	Tetradecanoic acid	$C_{14}H_{28}O_2$	−0.76*‡
7	14.499	1821	240	Hexadecanal	$C_{16}H_{32}O$	0.44
8	14.566	1827	234	Bakkenolide A	$C_{15}H_{22}O_2$	−0.42
9	14.846	1856	242	Pentadecanoic acid	$C_{15}H_{30}O_2$	−0.33
10	15.512	1922	–	Hexadecenoic acid derivative	$C_{16}H_{34}O_2$	−0.24
11	15.704	1940	254	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	−0.55*‡
12	15.914	1959	256	Hexadecanoic acid	$C_{16}H_{32}O_2$	−0.73*‡
13	16.702	2030	–	Heptadecanoic acid derivative	$C_{17}H_{34}O_2$	−0.39
14	17.023	2058	270	Heptadecanoic acid	$C_{17}H_{34}O_2$	−0.49
15	17.615	2108	254	5-Dodecyloxolan-2-one	$C_{16}H_{30}O_2$	0.87*†
16	17.973	2137	–	Octadecenoic acid derivative	$C_{18}H_{34}O_2$	−0.70*‡
17	18.268	2161	284	Octadecanoic acid	$C_{18}H_{36}O_2$	−0.74*‡
18	20.343	2321	–	Ethyl linoleate derivative	$C_{20}H_{40}O_2$	−0.37
19	20.902	2362	312	Eicosanoic acid	$C_{20}H_{38}O_2$	−0.58*‡
20	27.093	2813	410	Squalene	$C_{30}H_{50}$	−0.73*‡
21	27.814	2867	368	Cholesta-2,4-diene	$C_{27}H_{44}$	0.48
22	31.125	3123	386	Cholesterol	$C_{27}H_{46}O$	−0.18
23	31.208	3130	388	Cholestanol	$C_{27}H_{48}O$	0.88*†
24	32.513	3236	384	Cholest-5-en-3-one	$C_{27}H_{44}O$	0.62*†

Abbreviations: Cos, cosines between the 24 compounds and the first axe of the linear discriminant analysis (proxy of the contribution to the discriminant function); and RI calculated, Kovats' retention index calculated using a mixture of C_7–C_{40} alkanes with known RIs. *Compounds having a cosine > 0.5 (as an absolute value). †Compounds characterized sexually mature individuals. ‡Compounds characterized immature individuals.
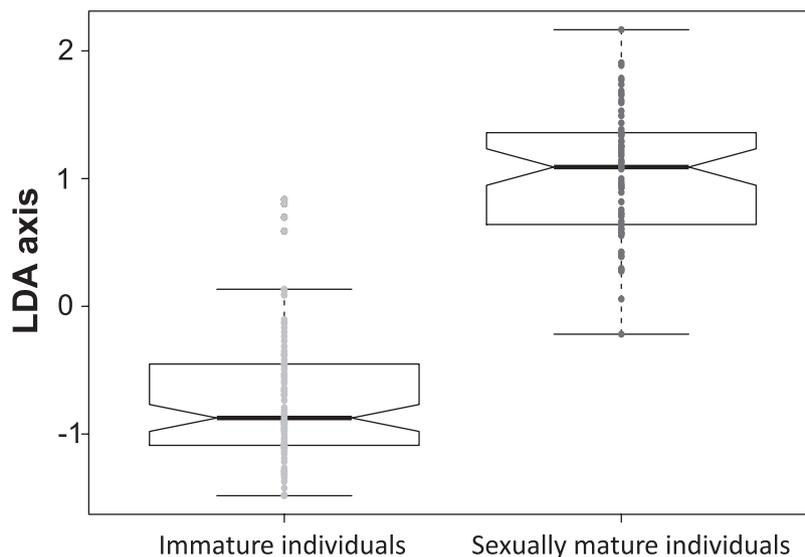
Figure 1. Box-plot of the score on the axis of the linear discriminant analysis (LDA) separating immature (light grey circles) from sexually mature individuals (dark grey circles).

individuals were relatively rich in fatty acids (e.g. dodecanoic acid, tetradecanoic acid, 9-hexadecanoic acid, hexadecanoic acid, octadecenoic acid derivative, octadecanoic acid and eicosanoic acid). They also presented a high relative abundance of a triterpene (squalene).

SCENT INFORMS ON SEX AND SOCIAL STATUS IN ADULT INDIVIDUALS

Of the 82 chemical compounds, 16 disappeared or were found in < 5% of sexually mature individuals. The PERMANOVA performed on the remaining 66 chemical compounds indicated a difference between social status (pseudo-F $F_{1,59}$ = 4.58, P = 0.003), but not between sexes (pseudo-F $F_{1,59}$ = 1.90, P = 0.096), and a significant interaction between sex and social status (pseudo-F $F_{1,59}$ = 3.23, P = 0.02). Of the 24 selected chemical compounds, five disappeared or were found in < 5% of sexually mature individuals [dodecanoic acid, (Z)-9-tetradecenoic acid, bakkenolide A, heptadecanoic acid derivative and ethyl linoleate derivative; Table 2]. The PERMANOVA performed on the 19 remaining compounds gave similar results to the one performed on the 66 compounds (social status, pseudo-F $F_{1,59}$ = 6.11, P = 0.031; sex, pseudo-F $F_{1,59}$ = 1.58, P = 0.18; and interaction, pseudo-F $F_{1,59}$ = 3.48, P = 0.032), indicating that no chemical information was lost using our procedure for compound selection.

The first two axes of the LDA performed on the 19 compounds separated adult individuals according to sex and social status well (Fig. 2). The first axis clearly opposed subordinate individuals and dominant individuals, whereas the second axis clearly opposed females and males. The LDA correctly assigned 87.50%
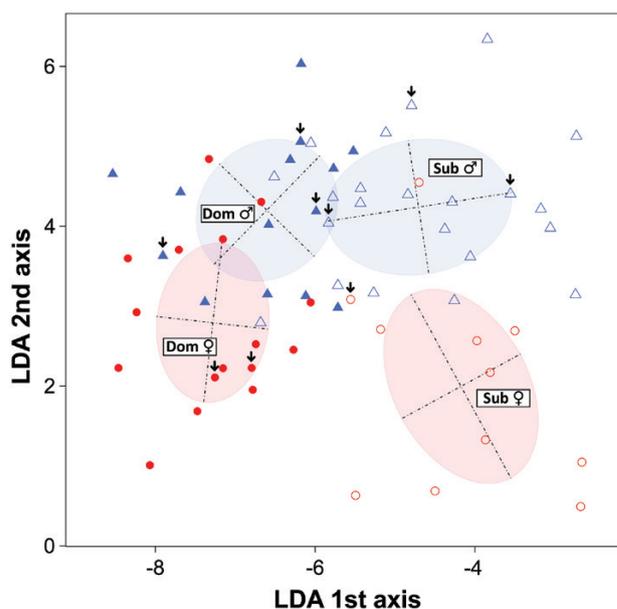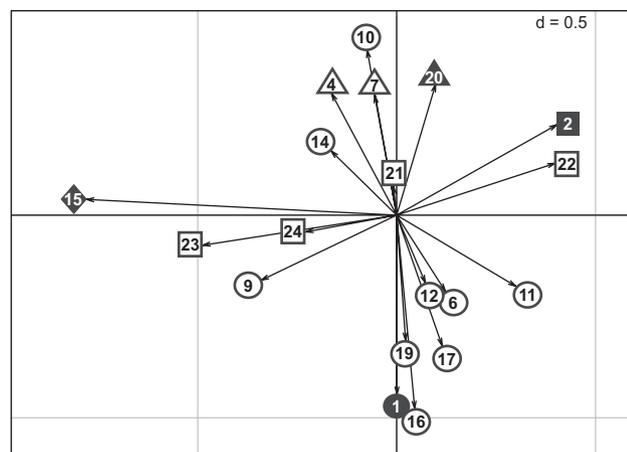

Figure 2. First factorial map of the linear discriminant analysis (LDA) separating adult individuals according to sex and social status. Sex: males are represented by blue triangles and females by red circles. Social status: dominant (Dom) individuals are represented by filled symbols and subordinate (Sub) individuals by open symbols. Three-year-old individuals (N = 9) are identified with an arrow.

of dominant females (only two dominant females were misclassified as dominant males) and 72.73% of subordinate females (three subordinate females were misclassified as subordinate males). In males, the LDA correctly assigned subordinate individuals much better than dominant individuals (90.91% and 64.29%,

respectively). Two dominant males were misclassified as dominant females, and three of them were misclassified as subordinate males, whereas only two subordinate males were misclassified, as a dominant male and a female. We found that anal gland secretions of sexually mature female Alpine marmots were relatively rich in fatty acids, piperidin-2-one (alkaloid) and cholestanol (steroid) compared with male individuals. Anal gland secretions of male Alpine marmots were instead relatively rich in hexadecenoic acid derivative (fatty acid), pentadecanal and hexadecanal (aldehydes), squalene (triterpene), cholesterol (steroid) and 3-phenylpropanoic acid (carboxylic acid) (Fig. 3). Furthermore, anal gland secretions of subordinate male Alpine marmots were relatively rich in cholesterol (steroid) and 3-phenylpropanoic acid (carboxylic acid) compared with dominant male individuals. Conversely, anal gland secretions of dominant female Alpine marmots were relatively rich in cholestanol (steroid) and pentadecanoic acid (fatty acid). One lactone (5-dodecyloxonan-2-one) seems to characterize dominant individuals, regardless of their sex (Fig. 3).

DISCUSSION

Our results indicated that the chemical composition of anal gland secretions strongly differed between sexually mature and immature Alpine marmots. When considering only adult Alpine marmots, our results also indicated that the chemical composition of anal gland secretions differed according to sex and social status.

SCENT OF SEXUAL MATURATION

We expected that the chemical composition of anal gland secretions would change after sexual maturity. This expectation was validated in the Alpine marmot. A change in scent chemical composition with sexual maturation has previously been reported in a variety of mammals (e.g. Asian elephants, *Elephas maximus*: Rasmussen *et al.*, 2002; giant pandas, *Ailuropoda melanoleuca*: Yuan *et al.*, 2004; owl monkeys, *Aotus nancymaae* and *Aotus* spp.: MacDonald *et al.*, 2008; Spence-Aizenberg *et al.*, 2018; mandrills, *Mandrillus sphinx*: Setchell *et al.*, 2010; Vaglio *et al.*, 2016; and greater sac-winged bats, *Saccopteryx bilineata*: Caspers *et al.*, 2011). Furthermore, Osada *et al.* (2009) have demonstrated that the composition of the urine of adult and prepubescent male rats differs significantly in the presence of a few specific compounds, and female rats are more attracted to the odour of adult males than that of prepubescent males, suggesting that the chemical composition of the urine of male rats is used by female rats to gain information about sexual maturity.

These differences in the chemical composition of anal gland scents between sexually mature and immature individuals might have several non-exclusive explanations. First, it is likely that these differences are a consequence of developmental processes. For example, these differences might reflect hormonal changes as individuals reach sexual maturity. Indeed, in many mammals, individuals reaching puberty show some modification of androgen concentrations (e.g. primates: Saltzman *et al.*, 2011; giraffes, *Giraffa camelopardalis*: Wolf *et al.*, 2018). Regarding testosterone, some testosterone-dependent volatile compounds found in the urine of male mice (*Mus musculus*; Novotny *et al.*, 1985) may modify male attractiveness, suggesting modification of the chemical composition by levels of testosterone (Jemiolo *et al.*, 1985). It is also possible that these differences are the consequence of other maturation processes. For example, these differences might be explained by a change in diet with age (Hagey *et al.*, 1997; Kwak *et al.*, 2008; Schaefer *et al.*, 2010). The diet of Alpine marmots is mainly composed of leaves and flowers, particularly of dicots (Massemin *et al.*, 1996; Garin *et al.*, 2008), but whether the diet changes with age is yet to be documented in the Alpine marmot. Finally, this could also be explained by modifications of anal gland microbial flora with ageing (e.g. van Dongen *et al.*, 2013; Leclaire *et al.*, 2014). Second, the scent of sexually mature individuals might signal to adults of the opposite sex that they are potential mates (Johansson & Jones, 2007) and to

Figure 3. Graphical representation of the cosines between the 19 compounds and the first two axes of the linear discriminant analysis separating adult individuals according to sex and social status. Each number represents a specific compound (see Table 2 for the corresponding compounds). Each symbol represents a chemical family: alkaloid (1; filled circle), carboxylic acid (2; filled square), fatty acids (6, 9, 10, 11, 12, 14, 16, 17, 19; open circles), aldehydes (4, 7; open triangles), lactone (15; filled diamond), triterpene (20; filled triangle) and steroids (21, 22, 23, 24; open squares).

same-sex adults that they are potential competitors (Ralls, 1971; Brown 1979; Gosling & Roberts, 2001). A change of scent chemical composition as individuals mature might also be a way for immature individuals to avoid aggression by dominant individuals, which may focus their aggression towards direct competitors (Rasmussen *et al.*, 2002).

SEX EFFECT

We expected that the chemical composition from anal gland secretions would differ between sexes. We found such a sex difference, but only in adult individuals; a result also found in mandrills (Setchell *et al.*, 2010). We found that the anal gland secretions of adult female Alpine marmots were relatively rich in cholestanol compared with adult male individuals, which was likewise found in anogenital gland secretions of female giant pandas (Yuan *et al.*, 2004). The anal gland secretions of adult male Alpine marmots were instead relatively rich in pentadecanal, and this compound has also been reported to be relatively more abundant in male than in female adult giant pandas (Yuan *et al.*, 2004).

Our results indicate that the chemical composition of anal gland scent might be used for sex recognition in the Alpine marmot. The ability of scent secretions to inform on sex is well documented in both eutherian mammals (e.g. hyena, *Crocuta crocuta*: Drea *et al.*, 2002; ring-tailed lemurs, *Lemur catta*: Hayes *et al.*, 2004; Scordato *et al.*, 2007; owl monkeys: MacDonald *et al.*, 2008; Eurasian otters, *Lutra lutra*: Kean *et al.*, 2011; brown bears, *Ursus arctos*: Rosell *et al.*, 2011; giant pandas: Yuan *et al.*, 2004) and monotremes (e.g. short-beaked echidna, *Tachyglossus aculeatus*: Harris *et al.*, 2012, 2014). Classically, sex recognition from olfactory cues has been suggested to play a role in sexual interactions such as mate location (Alberts, 1992), mate attraction (Haymann, 2006) and mate choice (Johansson & Jones, 2007). Furthermore, Alpine marmots are not sexually dimorphic in size and colour pattern; therefore, a sex-specific chemical scent might play an important role in sex recognition in this sexually monomorphic species. We cannot discard these hypotheses, but our results also support the prediction that sex recognition from olfactory cues might allow sexually mature individuals to target same-sex competitors during competitive interactions. In the Alpine marmot, intrasexual competition for dominant status still occurs after the mating season, with dominant individuals often being challenged and evicted during the whole of the active period. Accordingly, we found sex differences in anal gland secretions sampled after the mating season. More information is needed, however, to ascertain whether sex recognition from olfactory cues plays a role in intrasexual competition in the Alpine marmot.

SOCIAL STATUS EFFECT

We expected that the chemical composition of anal gland secretions would differ between dominant and subordinate individuals. This expectation was validated in the Alpine marmot, suggesting that the chemical composition of anal gland secretions might convey information on social status in both sexes in the Alpine marmot. This result was consistent with other studies in mammals (e.g. Hayes *et al.*, 2002; Burgener *et al.*, 2009; Setchell *et al.*, 2010).

This social status signature in Alpine marmot anal gland secretions might be attributable, in part, to social status being confounded with age in our samples. Indeed, the majority of subordinate individuals were 2 years old, whereas all dominant individuals were 3–12 years old. However, both dominant ($N = 5$) and subordinate ($N = 4$) individuals were found among 3-year-old individuals. Three-year-old dominant individuals had chemical profiles corresponding to that of older dominants, whereas 3-year-old subordinate individuals had chemical profiles that corresponded to that of 2-year-old subordinate individuals. Although the number of 3-year-old individuals was small, these results suggest that it is unlikely that the differences we found in chemical composition of anal gland secretions between dominant and subordinate individuals were attributable to an age effect alone.

Instead, we believe that these differences result from the outcome of intrasexual competition in Alpine marmots. Indeed, the competition for dominant status occurring between same-sex individuals within family groups of cooperative breeders results in the dominant individual suppressing the reproduction of non-evicted subordinate individuals. In Alpine marmots, there is a clear role of aggression by dominant individuals in the reproductive suppression of same-sex subordinate individuals (Arnold & Dittami, 1997; Hackländer *et al.*, 2003), whereas direct suppression through olfactory cues, as in other cooperative breeder species (e.g. inhibition of ovulation in daughters in cooperatively breeding primates; Ziegler, 2013), has not been evidenced. Aggression by dominant individuals results in high rates of stress hormones (e.g. corticosteroids), which in turn leads to low levels of testosterone in subordinate males and low levels of progesterone in subordinate females (Arnold & Dittami, 1997; Hackländer *et al.*, 2003). These physiological consequences of subordinate reproductive suppression may thus explain the differences in chemical composition we found between subordinate and dominant individuals of both sexes. However, reproductive suppression is not complete in subordinate males, which can sometimes gain extra-pair paternities (Goossens *et al.*, 1998; Cohas *et al.*, 2006). This incomplete reproductive suppression in male subordinates might explain the significant interaction between sex and social status in

PERMANOVAs and may thus explain why the difference in chemical composition of anal gland secretions between dominant and subordinate individuals was more pronounced in females than in males.

The differences in anal gland scent profiles between dominant and subordinate Alpine marmots might reduce overt conflicts among same-sex group adults, as suggested in other species (e.g. owl monkeys: MacDonald *et al.*, 2008). The scent of subordinate Alpine marmots might carry a non-threatening message to dominant individuals, which might then refrain from aggression. Likewise, field observations indicated that subordinate Alpine marmots often avoided coming too close to the same-sex dominant individuals; an avoidance also described in other species (e.g. Asian elephants; Rasmussen *et al.*, 2002).

CONCLUSION

Our results suggest that olfactory cues may serve in intrasexual competition in the Alpine marmot by informing others of sexual maturity, sex and dominance status. Our study supports the hypothesis that a change of scent chemical composition as individuals mature and become dominant could be a way in which to regulate the conflict (in both sexes) over access to dominance (and therefore reproduction) in cooperative breeders. Dominant individuals direct their aggressiveness towards individuals that represent the major threat and suppress the reproduction of subordinates (Young, 2009; Clutton-Brock & Huchard, 2013). However, to validate our conclusions, future behavioural experiments should investigate the response of individuals to the odour of conspecifics differing in sexual maturity, sex and social status.

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