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ORIGINAL PAPER

# An individual and a sex odor signature in kittiwakes? Study of the semiochemical composition of preen secretion and preen down feathers

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**Abstract** The importance of olfaction in birds' social behavior has long been denied. Avian chemical signaling has thus been relatively unexplored. The black-legged kittiwake provides a particularly appropriate model for investigating this topic. Kittiwakes preferentially mate with genetically dissimilar individuals, but the cues used to assess genetic characteristics remain unknown. As in other vertebrates, their body odors may carry individual and sexual signatures thus potentially reliably signaling individual genetic makeup. Here, we test whether body odors in preen gland secretion and preen down feathers in kittiwakes may provide a sex and an individual signature. Using gas chromatography and mass spectrometry, we found that male and female odors differ quantitatively, suggesting that scent may be one of the multiple cues used by birds to discriminate between sexes. We further detected an individual signature in the volatile and nonvolatile fractions of preen secretion and preen down feathers. These results suggest that kittiwake body odor may function as a signal associated with mate recognition. It further suggests that preen odor might broadcast the genetic makeup of individuals, and could be used in mate choice to assess the genetic compatibility of potential mates.

**Keywords** Kittiwake · Odor · Preen gland · Uropygial secretion · Individual signature

## Introduction

Birds protect their feathers by preening them with the secretions of the preen gland (also called uropygial gland;

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Stettenheim 1972). Among other functions, preen oil may protect the feathers from wear (Stettenheim 1972), aid in waterproofing (Giraudeau et al. 2010; Jacob and Ziswiler 1982), and protect against bacteria or dermatophytes (Jacob et al. 1997; Martin-Platero et al. 2006; Shawkey et al. 2003). These secretions also carry odors that may contribute to the chemical defense role of preen oil, by protecting birds against ectoparasites or predators (see review in Hagelin and Jones 2007). For example, when disturbed, the green woodhoopoe *Phoeniculus purpureus* releases a foul scented preen secretion that may provoke an avoidance reaction in predators (Burger et al. 2004).

Odors of preen secretion may differ greatly depending on the species, season, and/or sex (Haribal et al. 2005; Jacob and Ziswiler 1982; Mardon et al. 2010; Reneerkens et al. 2002; Soini et al. 2007). They have thus been suggested to act as intraspecific chemosignals, similar to those found in mammals (see review in Hagelin and Jones 2007, and Balthazart and Taziaux 2009). Antarctic prions *Pachyptila desolata*, for example, seem to recognize their mate by their individual odor, probably originating from the preen secretion (Bonadonna et al. 2007; Bonadonna and Nevitt 2004). Furthermore, in some species, female preen odor has been shown to influence the sexual behavior of males (Balthazart and Schoffeniels 1979; Hirao et al. 2009). In mallards *Anas platyrhynchos*, altered sexual behaviors were observed in males whose olfactory nerves had been sectioned (Balthazart and Schoffeniels 1979; see review in Balthazart and Taziaux 2009), and in domestic chicken *Gallus gallus domesticus*, anosmic males did not prefer control females over uropygial glandectomized females, whereas the preference was expressed by normal males (Hirao et al. 2009). Finally, preen odor has been hypothesized to influence mate choice by advertising the allelic status of an individual's MHC (Major Histocompatibility Complex) genes to potential mates (Freeman-Gallant et al. 2003; Soini et al. 2007). In other vertebrates such as primates, rodents, lizards, or fish, olfactory cues are implicated in the advertisement of genetic compatibility (Charpentier et al. 2008; Olsen et al. 1998; Olsson et al. 2003; Roberts and Gosling 2003; Singer et al. 1997; Wedekind and Penn 2000).

The black-legged kittiwake *Rissa tridactyla* is a particular suitable species to investigate the role of body scent in avian sexual behavior. Black-legged kittiwakes mate with genetically dissimilar individuals (Mulard et al. 2009), raising the question of how such discrimination is achieved. Vocal distance between individuals is not correlated with genetic distance, indicating that vocal cues probably give little information on individual genetic variation (Mulard 2007). As has been shown in other birds, kittiwakes can smell (Leclaire et al. 2009); thus, olfactory cues may be involved in genetic compatibility assessment, and carry an individual signature.

The nasal cavity of most mammals contains the main olfactory epithelium which detects small volatile compounds borne in air, and the vomeronasal organ which detects relatively nonvolatile compounds upon direct contact with the stimulus source (review in Brennan and Zufall 2006). Birds do not possess a vomeronasal organ (Bang and Wenzel 1985) or vomeronasal receptors (Shi and Zhang 2007), and it has been suggested that they cannot perceive non-airborne molecules by smell (Bonadonna et al. 2007). However, findings in mammals indicate that this functional division is not absolute and that nonvolatile compounds may also reach the main olfactory receptor neurons (Meredith and O'Connell 1988; Spehr et al. 2006). Several bird species including kittiwakes frequently allopreen, potentially exposing them to their conspecific's nonvolatile chemical compounds. This implies that relatively nonvolatile constituents present in body secretions of birds might act via the conventional sense of smell.

In this paper, the volatile and nonvolatile chemical composition of preen oil was studied to determine whether scent of kittiwakes may constitute an individual endogenous olfactory signature. The chemical composition of the down feathers surrounding the preen gland was further studied to analyze preen oil before and after it has resided on feather surfaces for a while.

## Materials and methods

### Preen secretions and feather samples

Samples were collected during the 2007 and 2008 breeding seasons, in a population of black-legged kittiwakes nesting on an abandoned US Air Force radar tower on Middleton Island (59° 26'N, 146° 20'W), Gulf of Alaska (Gill and Hatch 2002). The black-legged kittiwake is not known to be sexually dimorphic apart from a slight body size difference (Jodice et al. 2000). Once established, most pairs remain stable and return to the same nest year after year (Coulson 1966; Naves et al. 2006).

In 2008, samples were collected from 21 females and 20 males. Eighteen of these birds had also been sampled in 2007 ($n$=11 females and 7 males). Adult sexing was based on molecular sexing ($n$=17 birds), on copulation and courtship feeding during the pre-laying period ($n$=20 birds), or on skull length ($n$=4 birds, females <93 mm and males >98 mm; Jodice et al. 2000). Nest sites were observed twice daily to determine laying date.

Preen down feathers and preen oil were sampled for each bird that was captured. Preen down feathers were manipulated with new surgical gloves changed between samples and were cut from the down around the preen gland with steel scissors cleaned in ethanol between samples. Feathers

were stored in 2-ml vials with a PTFE-faced septum. Preen secretions were collected by gently pressing the base of the gland. The gland papilla was then gently rubbed with the tip of a glass capillary so that drops of secretions stuck around or inside the tip of the capillary. The end of the capillary was inserted in a 2-ml vial and broken off so that the back end of the capillary, which served as a handle while collecting, was discarded. Vials were then sealed with a PTFE-faced septum and frozen at −20°C immediately after sampling. Samples were shipped from the field to the chemical laboratory in a cool box filled with ice packs. They arrived refrigerated at the laboratory, and were then kept frozen at −20°C until analysis.

## Chemical analyses

Secretion and feather samples were analyzed from February to April 2009. Samples from the same individual or from individuals of the same sex were not analyzed together at the same time. Samples were immersed in 1 ml chloroform/nonadecane (internal standard, 2 μg/ml), agitated for 2 h at ambient temperature and then kept refrigerated until analysis. Samples were analyzed on a Varian 3900 gas chromatograph (Varian, Palo Alto, CA, USA), equipped with a flame-ionization detector and a J&W scientific DB-5MS (30 m× 0.25 mm, ID, film thickness 0.25 μm) capillary column. Helium was used as a carrier gas. The flame-ionization detector was operated at 300°C, and the injector was used at 300°C. Samples were injected in splitless mode. The oven was programmed as follows: 7°C/min from 50°C to 200°C and then 3°C/min to 290°C. The repeatability of the injection was estimated by the variation coefficient of the internal standard peak. Blanks were regularly interspersed throughout the sample analyses.

To determine the identity of compounds, gas chromatography–mass spectrometry was employed using a GC-MS Shimadzu QP2010 plus apparatus equipped with splitless injection and Supelco SLB-5ms capillary column. The scan range of the mass spectrometer was 38 to 600 *m/z*. The oven temperature program was the same as that above. Identification of compounds was based on mass spectral fragmentation pattern, using personal documents and various libraries and chromatographic retention index (NIST, Adams). Exact identification of each compound, through injection of commercial standards, was considered unimportant for the present study (as in Lawson et al. 2001; Mardon et al. 2010; Scordato et al. 2007).

## Statistical analysis

Compounds with less than 19 carbon atoms (i.e., compounds with a retention time lower than that of nonadecane [C19]) were considered volatile (Bonadonna et al. 2007; Zimmer and Zimmer 2008). As we could not control for the amount of secretion or feather collected, we did not rely on the absolute abundance of chromatogram peaks in our statistical analyses (as in Bonadonna et al. 2007; Jordan et al. 2010; Lawson et al. 2001; Macdonald et al. 2008; Mardon et al. 2010; Scordato et al. 2007). Rather, each peak was quantified as the relative proportion of the peak size to the overall volatile or nonvolatile area of the chromatogram. As volatile compounds were in very low proportion and represented only 0.7% of the overall chromatogram area, working on the nonvolatile chromatogram area was therefore quasi-similar as working on the total chromatogram area. Chromatograms were analyzed with the Varian Star integration software. Data were analyzed with SAS (SAS Institute Inc. 1999) and R statistical software (R Development Core Team 2008).

We first compared the chemical composition of preen secretion and feathers by running a principal component analysis (PCA) on the matrix of compounds and birds sampled in 2008. Then, PC variables were compared between secretion and feathers using mixed-effect linear models (proc MIXED in SAS). Sample type (secretion *vs.* feathers) and Sex were entered as fixed effects and bird identity (BirdID) as random factor. Multivariate ANOVAs could not be used to compare the two sample types as these tests do not allow entering random factors.

To compare the chemical composition of males and females, we used nonparametric MANOVAs using distance matrices (ADONIS in R). Then, *t* tests were performed on the 10 most abundant compounds to determine which main compounds contributed to the sex effect. Discriminant analyses (LDA in R) were performed on all compounds to determine whether sexes could be distinguished according to their preen gland and feather chemical compositions. Only the 2008 samples were considered for these analyses, as they are the more recent and so better preserved.

Samples of birds captured both in 2007 and 2008 ($n=18$ birds) were analyzed to determine the individual signature. In these analyses, we first reduced the number of compounds to reduce the number of dependent variables. In the nonvolatile fraction of preen secretions and preen down feathers, most compounds were present in all birds, and we retained compounds that comprised at least 1% of the nonvolatile chromatogram area (43 out of 126 compounds in preen secretion and 34 out of 129 compounds in preen feathers). In total, these compounds represented on average 86% (preen secretion) and 84% (feathers) of the nonvolatile chromatogram area. In the volatile fraction of preen down feathers, we retained only compounds that comprised on average 0.5% of the volatile chromatogram area (21 out of 81 compounds, representing 84% of the volatile chromatogram area on average). In the volatile fractions, many compounds were present in only a few

birds (59% of compounds were present in less than half the birds). As we could not determine whether these compounds were undetected because of the low concentration of compounds in secretion, we retained only the compounds that were present in more than 50% of individuals (40 out of 98 compounds, representing 89% of the volatile chromatogram area on average). We then ran a PCA on the matrix of selected compounds. We kept principal components (PC) that together accounted for at least 60% of the total variance. For those components, the variance among birds between years was estimated by entering the random factor BirdID in a mixed-effect linear models (proc MIXED in SAS). The Year random effect and the fixed factors "time before laying" and "sex" were also included in the models. The measure of repeatability of the olfactory signature of an individual bird was computed as the ratio of the variance among birds over years (i.e., BirdID random effect) to total variance (intraclass correlation coefficient [ICC]; as in Bonadonna et al. 2007). The blend of compounds was considered a potential individual olfactory signature if the ICC was higher than 50% and if the $P$ value associated with the BirdID random effect (Wald test) was lower than 0.05.

## Results

### Identification of chemical compounds

We identified 68 compounds (Table 1). Most of them were 2-methyl-substituted esterified fatty acids.

### Preen secretion *versus* preen down feathers

In the volatile fraction, 17 compounds were only found in down feathers and 14 were found in down feathers of most birds (i.e., in more than 75 % of birds), but rarely in preen secretion (i.e., in less than 25% of birds). We could not determine whether these compounds were absent or whether they were not detected because of a lower overall concentration of compounds in secretions. The chemical compositions of preen secretions and down feathers differed significantly (PC1: 15%, $F_{1,39}=135.67$, $P<0.0001$, when considering only compounds present in the two types of samples; Fig. 1). The two most abundant compounds were in higher proportion in down feathers than secretions (compound 3: $F_{1,39}=48.01$, $P<0.0001$ and compound 4: $F_{1,39}=46.59$, $P<0.0001$; Fig. 1), whereas the opposite was true for the other most abundant compounds (all $P<0.05$; except compound 5; Fig. 1). The random factor BirdID was nonsignificant in the analyses on PC1 but was significant in the analysis on the most abundant compounds (all $P<0.05$, except compound 3: $P=0.084$ and compound 4: $P=0.051$).

**Table 1** List of chemical compounds tentatively identified in preen feathers and secretions

| Number | Retention index | Compounds |
|---|---|---|
| 1 | 1083 | methyl octanoate |
| 2 | 1304 | methyl decanoate |
| 3 | 1489 | dodecanol |
| 4 | 1684 | tetradecanol |
| 5 | 1877 | hexadecanol |
| 6 | 2107 | undecyl 2-methylnonanoate |
| 7 | 2111 | dodecyl 2-methyloctanoate |
| 8 | 2165 | dodecyl octanoate |
| 9 | 2206 | undecyl 2-methyldecanoate |
| 10 | 2211 | tridecyl 2-methyloctanoate |
| 11 | 2217 | tetradecyl 2-methylheptanoate |
| 12 | 2226 | pentadecyl 2-methylhexanoate |
| 13 | 2259 | tetradec-2-yl 2-methyloctanoate |
| 14 | 2276 | tridecyl 2-methylnonanoate |
| 15 | 2304 | dodecyl 2-methyldecanoate |
| 16 | 2308 | tridecyl 2-methylnonanoate |
| 17 | 2313 | tetradecyl 2-methyloctanoate |
| 18 | 2319 | pentadecyl 2-methylheptanoate |
| 19 | 2357 | tridec-2-yl 2-methyldecanoate |
| 20 | 2368 | tetradecyl octanoate |
| 21 | 2402 | tridecyl 2-methyldecanoate |
| 22 | 2411 | pentadecyl 2-methyloctanoate |
| 23 | 2416 | tetradecyl 2-methylnonanoate |
| 24 | 2419 | hexadecyl 2-methyloctanoate |
| 25 | 2445 | tridecyl 2-methylundecanoate |
| 26 | 2450 | hexadecyl 2-methyloctanoate |
| 27 | 2456 | tetradec-2-yl 2-methyldecanoate |
| 28 | 2459 | hexadec-2-yl 2-methyloctanoate |
| 29 | 2473 | tridecyl 2-methylundecanoate |
| 30 | 2476 | pentadecyl 2-methylnonanoate |
| 31 | 2485 | hexadecyl 2-methyloctanoate |
| 32 | 2503 | tetradecyl 2-methyldecanoate |
| 33 | 2508 | pentadecyl 2-methylnonanoate |
| 34 | 2513 | tetradecyl 2-methyloctanoate |
| 35 | 2545 | tetradecyl 2-methylundecanoate |
| 36 | 2556 | pentadec-2-yl 2-methyldecanoate |
| 37 | 2569 | hexadecyl octanoate |
| 38 | 2584 | hexadecyl 2-methyloctanoate |
| 39 | 2601 | tridecyl 2 methyldodecanoate |
| 40 | 2604 | pentadecyl 2-methyldecanoate |
| 41 | 2614 | heptadecyl 2-methyloctanoate |
| 42 | 2641 | hexadecyl 2-methyldecanoate |
| 43 | 2646 | octadecyl 2-methyloctanoate |
| 44 | 2651 | hexadec-2-yl 2-methyldecanoate |
| 45 | 2652 | octadec-2-yl 2-methyloctanoate |
| 46 | 2672 | pentadecyl 2-methylundecanoate |
| 47 | 2679 | heptadecyl 2-methylnonanoate |
| 48 | 2700 | pentadecyl 2-methylundecanoate |

**Table 1** (continued)

| Number | Retention index | Compounds |
|---|---|---|
| 49 | 2704 | hexadecyl 2-methyldecanoate |
| 50 | 2709 | heptadecyl 2-methylnonanoate |
| 51 | 2715 | octadecyl 2-methyloctanoate |
| 52 | 2746 | hexadecyl 2-methylundecanoate |
| 53 | 2759 | heptadec-2-yl 2-methyldecanoate |
| 54 | 2778 | octadecyl octanoate |
| 55 | 2799 | pentadecyl 2-methyldodecanoate |
| 56 | 2805 | heptadecyl 2-methyldecanoate |
| 57 | 2809 | octadecyl 2-methylnonanoate |
| 58 | 2831 | pentadecyl 2-methyltridecanoate |
| 59 | 2845 | heptadecyl 2-methyl undecanoate |
| 60 | 2877 | octadecyl 2-methyldecanoate |
| 61 | 2896 | hexadecyl 2-methyldodecanoate |
| 62 | 2929 | hexadecyl 2-methyltridecanoate |
| 63 | 2932 | heptadecyl 2-methyldodecanoate |
| 64 | 2971 | octadecyl 2-methylundecanoate |
| 65 | 2994 | heptadecyl 2-methyldodecanoate |
| 66 | 3028 | heptadecyl 2-methyl tridecanoate |
| 67 | 3039 | octdecyl 2-methyl dodecanoate |
| 68 | 3062 | heptadec-2-yl 2-methyltridecanoate |

Compounds with the same name are structural isomers

In the nonvolatile fraction, all compounds were common to preen secretion and down feathers. However, the chemical compositions of preen secretion and down feathers differed quantitatively (PC1: 29%, $F_{1,37}$=7.16, $P$=0.011, PC2: 16%: $F_{1,37}$=324.11, $P$<0.0001). The difference in the proportion of each compound between down feathers and secretions was correlated with the retention time (Spearman correlation: $r^2$=0.49, $P$<0.0001, $n$=4180; Fig. 1). The lower the molecular weight of the compound, the lower the proportion of this compound in down feathers compared to secretions (Fig. 1). The chemical composition of down feathers and secretions was more similar within an individual than among individuals (BirdID random effect; PC1: $Z$=3.77, $P$<0.0001, PC2: $Z$=3.75, $P$<0.0001).

## Sex effect

No single compound was systematically present in one sex and absent in the other. However, the volatile and nonvolatile fractions of the preen secretions ($F_{1,39}$=3.07, $P$=0.003 and $F_{1,37}$=3.90, $P$=0.011, respectively; Fig. 2) and down feathers ($F_{1,36}$=3.15, $P$=0.014 and $F_{1,38}$=3.41, $P$=0.021, respectively) differed quantitatively between males and females. A discriminant analysis performed on the volatile or nonvolatile compounds of preen secretion and down feathers assigned more than 90% of individuals to the correct sex (volatile secretion 92.7%, nonvolatile secretion 92.5%, volatile feathers 94.7%, and nonvolatile feathers 95%).

In the volatile fraction of preen secretions, two out of the ten most abundant compounds were significantly in lower proportion in males than females (compound 3: $t_{39}$=2.81, $P$=0.0077 and compound 4: $t_{39}$=3.59, $P$=0.0009; Fig. 2). These two compounds were the two main volatile compounds, and were also in higher proportions in females than males in preen down feathers ($t_{38}$=2.24, $P$=0.031 and $t_{38}$=2.56, $P$=0.015, respectively).

In the nonvolatile fraction of preen secretion, seven out of the ten most abundant compounds were in lower proportions in males than females (all $P$<0.015; Fig. 2). These seven compounds were also in higher proportions in females than males in the nonvolatile fraction of preen down feathers (all $P$<0.02 except for compound 65: $P$=0.07).

## Individual signature

In the volatile fraction of preen secretions, the first five components (PC1–PC5) accounted for 20%, 16%, 10%, 7%, and 6% of the variation in the proportions of compounds. As a factor explaining variation in PC1 among birds between years, the BirdID random factor was significant ($Z$=2.00, $P$=0.023) and accounted for 27% of the total variation. Among the identified compounds, PC1 was correlated to compound 3 ($P$=0.014, $r$=−0.41). The BirdID random factor was nonsignificant in the analyses of PC2, PC3, PC4, or PC5.

In the volatile fraction of down feathers, the first three components accounted for 30%, 16%, and 14% of the variation in the proportion of the most abundant compounds. As an explanation of variation in PC3 among birds over years, the BirdID random factor was significant ($Z$=1.83, $P$=0.034) and accounted for a large fraction of the total variation (ICC=50%). PC3 also depended upon the sexes ($F_{1,11}$=12.48, $P$=0.0047). PC3 was significantly correlated to none of the identified compounds. The BirdID random factor did not explain variations in PC1 or PC2 significantly.

In the nonvolatile fraction of preen secretions and down feathers, PC1 accounted for 43% and 46% and PC2 for 28% and 27%, respectively, of variation in the proportions of the main compounds. The BirdID effect on the variation in PC1 was significant ($Z$=2.30, $P$=0.011 and $Z$=2.19, $P$=0.014 respectively, Fig. 3) and accounted for large fractions of the total variation (ICC=58% and 53% respectively). The interaction Sex×Time-before-laying was also significant ($F_{1,18.5}$=9.08, $P$=0.0073, Fig. 4 and $F_{1,17.1}$=4.49, $P$=0.049). PC1 increased in females as laying date approached, but it did not vary in males. The year random effect was not significant (preen secretion: $Z$=0.67, $P$=0.25 and down

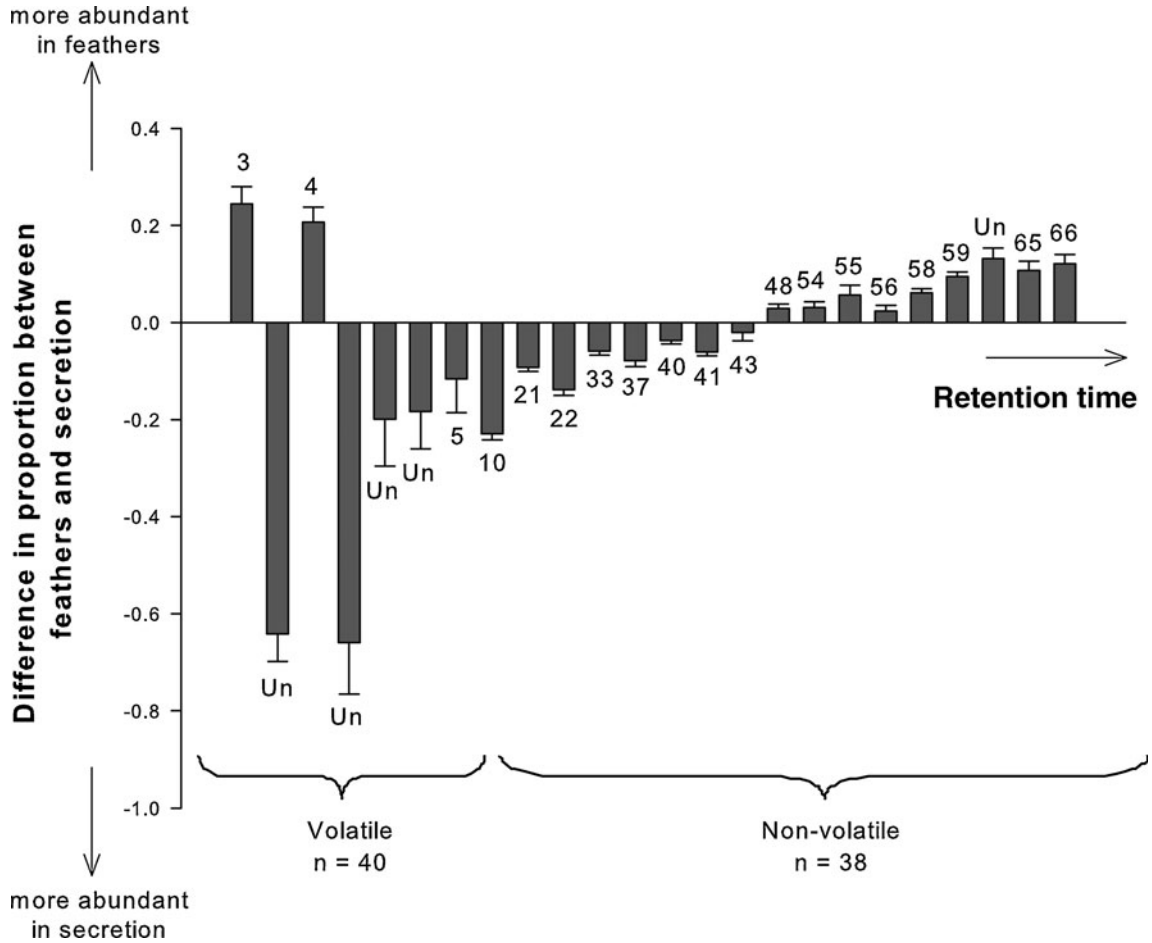


**Fig. 1** Difference between feathers and secretion in compound proportion [i.e., (proportion in feather−proportion in secretion)/average proportion]. Shown are compounds representing at least 2% of the overall chromatogram, and *numbers* are those listed in Table 1. *Un* unidentified compounds

feathers: $Z$=0.67, $P$=0.25, Fig. 3). PC1 in secretion and down feathers correlated strongly with compounds 10, 21, 22, 32, 33, 37, 40, 41, 43, 48, 55, 56, 57, 58, 65, 67, and 68 (all $P$<0.0001 and $r$>+0.73, except compound 37: $r$<−0.86). The BirdID effect on the variation in PC2 was not significant ($Z$=1.26, $P$=0.10 and $Z$=0.47, $P$=0.32, Fig. 3). PC2 decreased in males and females as laying date approached (preen secretions: $F_{1,28.3}$=4.64, $P$=0.040 and down feathers: $F_{1,27.8}$=6.09, $P$=0.020).

## Discussion

We characterized the chemical compounds of preen gland secretion and down feathers surrounding the preen gland in black-legged kittiwakes. We mainly found 2-methyl-substituted esterified fatty acids. This result is consistent with previous investigations of uropygial secretion contents in kittiwakes (Jacob and Zeman 1972). Large wax esters are also present in preen oil of most other avian groups (e.g., Bohnet et al. 1991; Dekker et al. 2000; Jacob and Ziswiler 1982; Mardon et al. 2010).

We investigated whether those secretions contained an individual signature, that is, whether secretions were individual-specific despite potential variation due to physiological or environmental factors. Although well explored in mammals (e.g., Lawson et al. 2000; Penn et al. 2007; Scordato et al. 2007; Smith et al. 2001), the existence of an individual odor signature in birds has only been demonstrated in few species (i.e., Antarctic prions *P. desolata* and blue petrels *Halobaena caerulea*, Bonadonna et al. 2007; Mardon et al. 2010; dark-eyed juncos *Junco hyemalis*, Whittaker et al. 2010). Our results show that in kittiwakes, too, an odor signature exists in the nonvolatile and volatile fractions of secretion and down feathers. Individual signatures may result from environmental microvariations between individuals and/or from genetic factors. For instance, in humans or mice, odor is influenced by the

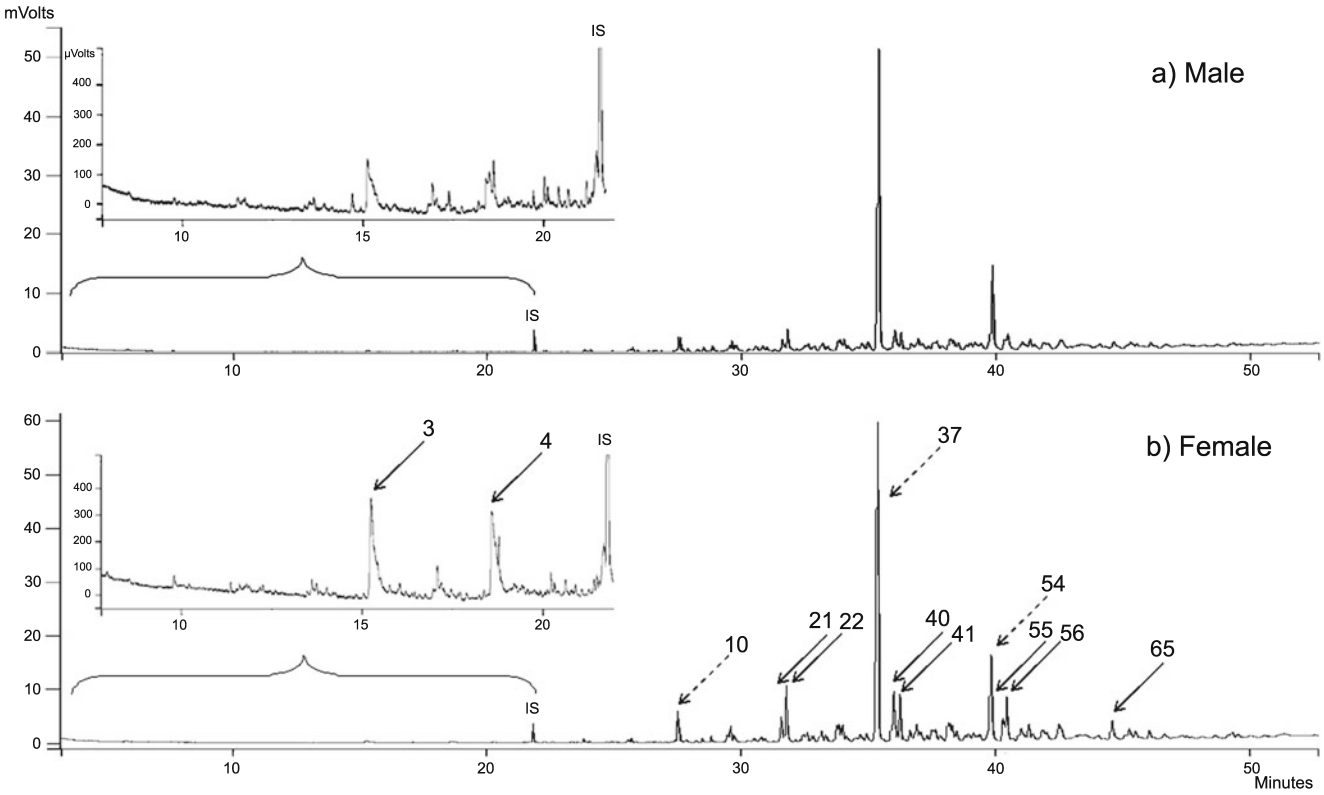


**Fig. 2** Representative FID chromatograms for preen secretion of male (**a**) and female (**b**) kittiwakes. Chromatograms representing volatile compounds are *enlarged* to visualize peaks. *Solid arrows* indicate the main compounds that were in significantly higher proportions in females than males. *Dashed arrows* indicate the main identified compounds that were not in different proportion between males and females. *IS* internal standard

highly polymorphic MHC loci, in interaction with microflora composition (James et al. 2004; Toivanen et al. 2001; Wedekind et al. 1995).

In kittiwakes, males and females were not known to be sexually dimorphic apart from a slight body size difference. Here, we showed that males and females have a different preen wax composition and that sexes could be distinguished according to it. Similar sexual differences have been demonstrated in dark-eyed junco (Soini et al. 2007), mallards *A. platyrhynchos* (Jacob et al. 1979), hoopoe *Upupa epops* (Martin-Platero et al. 2006), blue petrels and Antarctic prions (Mardon et al. 2010), and some sandpiper species (Scolopacidae, Reneerkens et al. 2007), and may partly be due to steroid sex hormones (Bohnet et al. 1991; Douglas et al. 2008). Additionally, variations in diet (Havlicek and Lenochova 2008) or in microflora (Rennie et al. 1990), possibly differing between sexes, are known to affect body odors in mammals. Many mammals discriminate between male and female odors (e.g., Drea et al. 2002; White et al. 2004), which can be used in territorial or sexual contexts (Cloe et al. 2004; Johnston 1986). In birds, the study of body odor is expanding (review in Balthazart and Taziaux 2009; Caro and Balthazart 2010; Hagelin and Jones 2007), but the use of volatile cues in sex discrimination has been investigated only in budgerigars *Melopsittaccus undulates*, which seem to distinguish the sex of a conspecific by the scent of preen secretion (Zhang et al. 2010; but see Mardon et al. 2011b), and in Antarctic prions which do not (Bonadonna 2009).

We found that the composition of preen secretion and preen down feathers varies over the pre-laying period, especially in females. Sex steroid hormones, which may promote synthesis of chemical scents in vertebrates (Bohnet et al. 1991; Kikuyama et al. 2005; Yamamoto et al. 1996), vary during egg formation in females (Sockman and Schwabl 1999; Williams et al. 2004). Such changes in body scent have been shown to facilitate reproductive behaviors in several mammals (Halpern et al. 1998; Miller and Maner 2010). In birds, it has been suggested that body scent may influence sexual behavior in mallards and chickens (Balthazart and Schoffeniels 1979; Hirao et al. 2009). Production of diester wax increases in female mallards during the breeding season, and sexual behavior

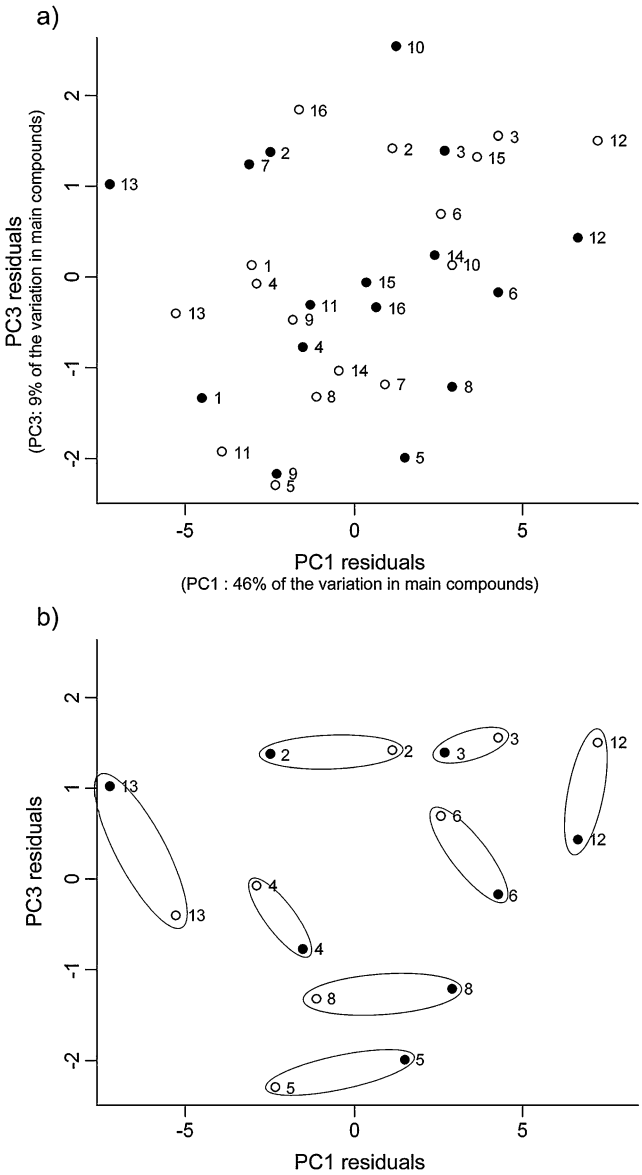


**Fig. 3** **a** Profile of nonvolatile preen feather compounds of 16 birds (as *numbered*) over 2 years (*open vs. filled symbols*) as described by the residuals of a model with PC1 or PC3 as the dependant variable, and Year and Time-before-laying × Sex as fixed effects. We used PC3 and not PC2 because the random individual effect was stronger in the PC3 analyses ($P$=0.07). **b** Same representation as in **a** but with only a selection of birds to better show clustering

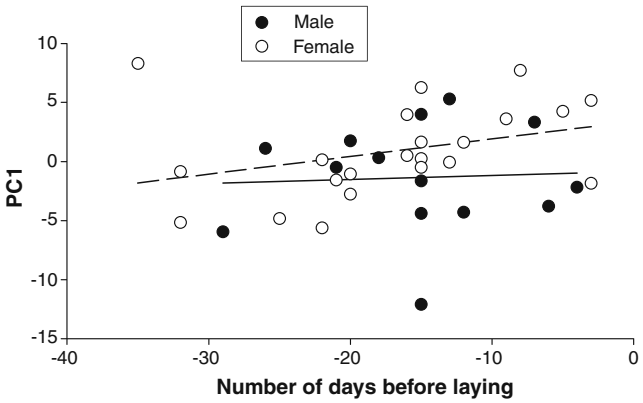


**Fig. 4** PC1 according to the time between capture and laying in males (*filled symbols*, *solid line*) and females (*open symbols*, *dashed line*). PC1 derived from PCA analysis of the variation in the proportion of nonvolatile preen secretion compounds

is inhibited in male mallards with olfactory nerve sectioned (Balthazart and Schoffeniels 1979; Jacob et al. 1979; see review in Balthazart and Taziaux 2009).

Results showed that the chemical compositions of preen secretion and preen down feathers differed quantitatively. The proportion of small compounds was found to be lower in down feathers than in secretion. Small compounds volatilize more rapidly than heavy compounds. This may explain why they are found in lower proportions on feathers. Furthermore, oxidation and feather-degrading bacteria may cause the differences observed between the two kinds of samples (Douglas 2008). Degradation may accelerate once preen secretion is spread over the whole plumage where it mixes with secretions of other glands such as sebaceous secretions from the skin (Hagelin and Jones 2007). For instance, in blue petrels, 85% of the plumage compounds are present in preen secretion (Mardon et al. 2011a), and in wood pigeons, only 6.7% of the whole-plumage lipids were considered to originate from the uropygial contents (Jacob and Grimmer 1975, in Jacob and Ziswiler 1982). Preen secretion aids in waterproofing (Giraudeau et al. 2010; Jacob and Ziswiler 1982) and is thus largely spread on the plumage of seabirds. Consequently, sex and individual signatures contained in blue petrels' secretion are also found in plumage compounds (Mardon et al. 2011a). Another sampling protocol (e.g., Douglas 2006) would however be necessary to determine precisely the whole body odor of kittiwakes.

In conclusion, our study suggests the existence of two odor signatures in kittiwakes: a sex and an individual signature. These results suggest that kittiwake body odor may function as a signal associated with individual recognition and mate choice. Chemical individual signature has been shown to result partly from genetic factors in several species (Wedekind and Penn 2000; Yamazaki et al. 1999). Kittiwakes preferentially mate with genetically unrelated individuals (Mulard et al. 2009). Our results may therefore indicate that they might use body odor to assess the genetic compatibility of potential mates. Further studies, including correlations of odor profiles with genetic characteristics as well as behavioral observations and experiments, are needed to determine the role of body odor in mate choice in this species.

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