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Seasonal Variation in Volatile Compound Profiles of Preen Gland Secretions of the Dark-eyed Junco (*Junco hyemalis*)

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Abstract Quantitative stir bar sorptive extraction methodology, followed by gas chromatography-mass spectrometry (GC-MS) and element-specific atomic emission detection (AED) were utilized to analyze seasonal changes in volatile components of preen oil secretions in *Junco hyemalis*. Juncos were held in long days to simulate breeding conditions, or short days to simulate nonbreeding conditions. Linear alcohols (C_{10}–C_{18}) were the major volatile compounds found in preen oil, and in both sexes their levels were higher when birds were housed on long as opposed to short days. Methylketones were found at lower levels, but were enhanced in both sexes during long days. Levels of 2-tridecanone, 2-tetradecanone, and 2-pentadecanone were also greater on long days, but only in males. Among carboxylic acids (C_{12}, C_{14}, and C_{16}), linear but not branched acids showed some differences between the breeding and nonbreeding conditions, although the individual variation for acidic compounds was large. Qualitatively, more sulfur-containing compounds were found in males than females during the breeding season. Functionally, the large increase in linear alcohols in male and female preen oil during the breeding season may be an indication of altered lipid biosynthesis, which might signal reproductive readiness. Linear alcohols might also facilitate junco odor blending with plant volatiles in the habitat to distract mammalian predators. Some of the volatile compounds from preen oil, including linear alcohols, were also found on the wing feather surface, along with additional compounds that could have been of either metabolic or environmental origin.

Keywords Avian volatile compounds · Preen oil · *Junco hyemalis* · Stir bar sorptive extraction · Gas chromatography-mass spectrometry

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Introduction

In birds, the uropygial (also called "preen") gland produces lipids, much like those produced by mammalian sebaceous glands. These lipids are spread over the feathers as the bird uses its bill to preen. As an example, the junco first rubs its bill against the gland located on its rump, and then applies the secretions as it runs individual feathers between its upper and lower mandibles (Schrock, personal observation). Among other functions, the oils may protect the feathers from wear (Stettenheim, 1972), aid in waterproofing (Elder, 1954; Fabricius, 1959; Elowson, 1984), or protect against dermatophytes (Jacob et al., 1997) including lice (Moyer et al., 2003).

Traditionally, the composition of preen oil gland secretions has focused on nonvolatile compounds and has been investigated by hydrolyzing the waxes (mostly nonvolatile compounds) under basic conditions into acids, acid esters, and alcohols. Long-chain and branched acids and their esters were thus found to be the main components in hydrolyzed wax collected from the preen gland of the mute swan (*Cygnus olor*) (Odham, 1965) and also from waterfowl belonging to the avian family *Anatidae* (Odham, 1967). Even carbon-numbered long-chain alcohols were also recovered from hydrolyzed preen wax in a shorebird, the red knot (*Calidris canutus*) (Dekker et al., 2000).

Several investigations of the waxy composition of preen gland secretions have compared the nonbreeding and breeding seasons and reported shifts from monoester to diester waxy components in sandpipers (*Scolopacidae*) and in male and female Mallard ducks (*Anas platyrhynchos*) (Bohnet et al., 1991; Reneerkens et al., 2002). It was hypothesized for the sandpipers (*Scolopacidae*) that a shift from more volatile monoester preen waxes in winter to less volatile diester preen waxes in spring and summer may reduce the "odor" of the bird's feathers causing it to transfer less of this odor to the nest when incubating, and thus making the nest less noticeable to olfactorily sensitive mammalian nest predators (Reneerkens et al., 2002). Alternatively or in addition, the diester waxy preen oil during summer may alter the sandpiper's appearance, perhaps by influencing UV reflectance or other aspects of visual attractiveness, and thus play a role in mate choice (Reneerkens et al., 2002). Bohnet et al. (1991) suggested that compounds from the preen gland, when spread over the feathers, may also influence mate choice through a potential sex pheromone action. Preen oil may even have a role in advertising the allelic status of an individual's MHC genes to potential mates via a particular odor (Freeman-Gallant et al., 2003).

Recently, in studies with the great snipe (*Gallinago media*), which is a lekking bird species, a correlation between the MHC lineage and a mating success has been confirmed (Ekblom et al., 2004). The olfaction-mediated functions in birds, more than was previously appreciated, are plausible in light of new evidence for a stronger role for olfaction in passerines and other avian orders (see Roper, 1999 for a review).

Junco hyemalis is a classic species for the study of seasonality, as it was the first wild species shown to respond to changes in day length (summarized in Nolan et al., 2002). *J. hyemalis* is a socially monogamous, ground-nesting bird. Plasma testosterone levels have been reported to vary seasonally in both males and females (Schoech et al., 1998; Ketterson et al., 2005). Although the female builds the nest and does all the incubation, the male also provides parental care by delivering food to the young in the nest (Nolan et al., 2002).

To date, relatively few studies of any bird species have reported the composition of the volatile fraction of the preen oil. As with the contents of waxy compounds, the composition of volatile compounds reported in preen gland secretions and feathers also appears to differ greatly depending on the bird species. A "citrus odor" found on the feathers of the crested

auklet (*Aethia cristatella*) has been found to contain a group of aldehydes (hexanal, octanal, decanal, Z-4-decenal, and 12-carbon unsaturated aldehyde) that act as the major volatile chemosignals (Douglas et al., 2001). It was originally suggested that these aldehydes are capable of repelling invertebrate species, bacteria, and fungi, and consequently might signal an individual's health and vigor in the mate selection process.

A more recent study has shown that the crested auklet feather aldehydes are effective as mosquito repellants (Douglas et al., 2005). In addition, Hagelin et al. (2003) established that the "tangerine odor" in volatile secretions (octanal and *cis*-4-decenal) of the crested auklet feathers act as social odors to present to conspecifics. Partner-specific olfactory recognition was also observed between Antarctic prions (*Pachiptila desolata*) by Bonadonna and Nevitt (2004), but the specific compounds involved were not identified. Foul-smelling preen gland secretions (consisting of acids, aldehydes, ketones, amines, and dimethyl sulfide) in the green woodhoopoe (*Phoeniculus purpureus*) serve as a multifunctional tool, repelling predators and warding off feather-degrading bacteria (Burger et al., 2004).

In this paper, we report on the volatile and semivolatile chemical composition of the preen gland secretions of male and female dark-eyed juncos (*J. hyemalis*) during the breeding and nonbreeding seasons. Sorptive stir bar extraction methodology (Soini et al., 2005) with gas chromatography-mass spectrometry (GC-MS) was utilized in the investigation of the preen oil compositions. A novel *in situ* stir bar sampling method (Soini et al., 2006) was also applied to study volatile compounds on the surface of the wing feather.

Methods and Materials

Preen Oil Collection Sample secretions were collected from captive juncos in breeding and nonbreeding conditions. The juncos were originally captured near Pembroke, VA, USA and were held thereafter at the Kent Farm Bird Observatory in Bloomington, IN, USA. Their diet consisted of millet seed and a pureed mixture of dog chow, hard-boiled eggs, and carrots supplemented with vitamins and mealworms, and was available *ad libitum*. A total of 20 individuals were used, including 10 males and 10 females, and each individual was sampled first under short days and then under long days in a repeated measures design. Both short- and long-day conditions were maintained for 8 wk before the sample collection. The short-day birds (8L:16D) are referred to as "nonbreeding," whereas the long-day birds (16L:8D) are referred to as "breeding." In fact, neither group bred, but the long-day birds exhibited indicators of reproductive readiness, i.e., males developed large cloacal protuberances and females developed brood patches (defeathered areas on the ventral surface that transfer heat to developing embryos). Air temperature also differed by season: nonbreeding, short-day birds were held at −2–4°C, whereas in the breeding-season, long-day birds were held on at 18–20°C. Temperature conditions are typical temperatures juncos would experience in the wild population at Mountain Lake Biological Station in Virginia during the normal seasonal cycle.

Preen gland contents were collected by gently squeezing the area around the gland until a small amount of oil was discharged (Kolattukudy et al., 1987). The preen oil was then collected with a 100-μl microcapillary glass pipette that was weighed before and after sampling to determine how much was collected. The experimental procedures were approved by the Bloomington Institutional Animal Care and Use Committee of Indiana University. The samples (1.3–1.8 mg) were frozen at −20°C for the gas chromatography-

mass spectrometry (GC-MS) and gas chromatography-atomic emission detection (GC-AED) analyses.

Reagents and Materials All compound identifications were verified through authentic standards. The standard compounds and ammonium sulfate (99% +) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). The stir bars (Twister™, 10 mm, 0.5-mm film thickness, 24-μl polydimethylsiloxane, PDMS volume) used for the sorptive extraction were purchased from Gerstel GmbH (Mülheim an der Ruhr, Germany).

Preparation of Preen Oil Samples Volatile and semivolatile compounds were extracted from the preen oil samples by placing a 100-μl glass capillary containing the sample in a 20-ml capped glass vial with 2 ml of water (high-purity OmniSolv® water from EM Science, Gibbstown, NJ, USA), 100 mg of ammonium sulfate, and a Twister™ stir bar. As an internal standard, 8 ng of 7-tridecanone (Aldrich) was added in 5 μl volume of methanol to each vial. The stirring speed was 800+ rpm on the Variomag Multipoint HP 15 stirplate (H+P Labortechnic, Oberschleissheim, Germany) for a 60-min extraction time. Before extraction, all glassware was washed with acetone and dried at 80°C. After extraction, the stir bars were rinsed with a small amount of distilled water, dried gently on the paper tissue, and placed in the TDSA autosampler tube (a product of Gerstel) for the GC or GC-MS analysis.

Collection of Volatile Compounds from Surface of the Wing Feather A rolling stir bar method (Soini et al., 2006) was applied in the collection of wing feather volatiles from the live bird wings. A wing feather of a single junco was sampled, rolling a stir bar gently over about 4 cm^2 surface area of its length. Sample-containing stir bars were subsequently stored in a refrigerator. The analyses were performed within 48 hr after collection. Stir bars were placed in the thermal desorption autosampler (see the description of equipment) without any further sample manipulation.

Analytical Instruments The GC equipment for quantitative analyses consisted of the Agilent 6890N gas chromatograph connected to a 5973i MSD mass spectrometer (Agilent Technologies, Inc., Wilmington, DE, USA) with the Thermal Desorption Autosampler and Cooled Injection System (TDSA-CIS 4 from Gerstel). Positive electron ionization (EI) mode at 70 eV was used with the scanning rate of 2.47 scans/sec over the mass range of 40–350 amu. A MSD transfer line temperature was set at 280°C. The ion source and quadrupole temperatures were set at 230°C and 150°C, respectively. The separation capillary was DB-5MS (20 m×0.25 mm, i.d., 0.25 μm film thickness) from Agilent (J&W Scientific, Folsom, CA, USA).

Samples were thermally desorbed in a TDSA automated system, followed by injection into the column with a cooled injection assembly, CIS-4. TDSA operated in a splitless mode. Temperature program for desorption was 20°C (0.5 min), then 60°C/min to 250°C (3 min). Temperature of the transfer line was set at 280°C. CIS was cooled with liquid nitrogen to −80°C. After desorption and cryotrapping, CIS was heated at 12°C/sec to 280°C, with the hold time of 10 min. CIS inlet was operated in the solvent-vent mode, a vent pressure of 14 psi, a vent flow of 50 ml/min, and a purge flow of 50 ml/min. The temperature program in the GC operation was 50°C for 2 min, then increasing to 200°C at the rate of 3°C/min (hold time, 10 min). The carrier gas head pressure was 14 psi (flow rate, 1.1 ml/min at the constant flow mode).

Element-selective compound profiling was performed using a GC 6890 instrument equipped with an Atomic Emission Detector (AED, model G2350A) from Agilent Technologies (Wilmington, DE, USA) and a Thermal Desorption Autosampler-Cooled Injection System (TDSA-CIS-4 from Gerstel). The separation capillary was an HP-5MS (30 m× 0.25 mm, i.d., 0.25 μm film thickness) from Agilent. Samples were thermally desorbed in a TDSA automated system, followed by injection into the column with a cooled injection assembly under the same conditions as described above for the GC-MS analysis, except that the CIS was cooled with liquid nitrogen to −60°C. The temperature program in the GC operation was 50°C for 2 min, then increasing to 200°C at the rate of 3°C/min. The final temperature was held for 12 min. The carrier gas head pressure was 14 psi (flow rate, 1.2 ml/min). The GC unit was operated at the constant flow mode. The emission lines for carbon (193 nm), sulfur (181 nm), and nitrogen (174 nm) were monitored during the atomic plasma emission detection.

Quantitative Comparisons A total of 24 samples were quantitatively analyzed by GC-MS, six from each group (breeding and nonbreeding females and males). Four other samples from each of the 4 groups were qualitatively screened for the sulfur compounds by the GC-AED method. Of the approximately 100 compounds detected in chromatographic profiles of the preen oil by GC-MS, about 40 were tentatively identified. All major compounds were positively identified through comparison of their mass spectra and retention times to those of standard substances. Peak areas of the identified compounds were used to make quantitative comparisons between the long- and short-day groups of each sex. Peak areas were integrated either from the TIC (total ion current) profiles or from the postrun, selected ion current (SIC) profiles at *m/z* 55, *m/z* 58, and *m/z* 60. Peak areas of the internal standard were integrated from the *m/z* 113 profiles. Peak areas of the compounds of interest were normalized by dividing each peak area by that of the internal standard in corresponding runs. Reproducibility of the internal standard peak area was 13% (relative standard deviation, RSD, $N=12$).

Statistical Analyses Statistical comparisons of the normalized peak areas were calculated by using nonparametric, two-tailed Mann–Whitney matched pairs *U* tests for seasonal comparisons within sex and simple Mann–Whitney *U* tests for sex comparisons within a season. Statistically significant values of $P<0.05$ were accepted.

Results and Discussion

The volatile compound GC-MS profiles (collected under the scan mode over 40–350 amu) were complex when viewed as total ion current chromatograms (TICs). The profiles consisted of the major components (peak intensity 50–2,000 times over the detection limit), along with a large number of minor components (peak intensity 10–50 times over the detection limit). Because of this degree of complexity, not all compounds were fully resolved in the GC separation. Figure 1 illustrates a compound pattern that is representative of the "raw" volatile compound profiles. To facilitate more precise comparisons of the peak area integrations, we employed a postrun modification of the TIC profiles known as selected ion current (SIC) profiles, which allowed us to view the data "one ion" at a time.

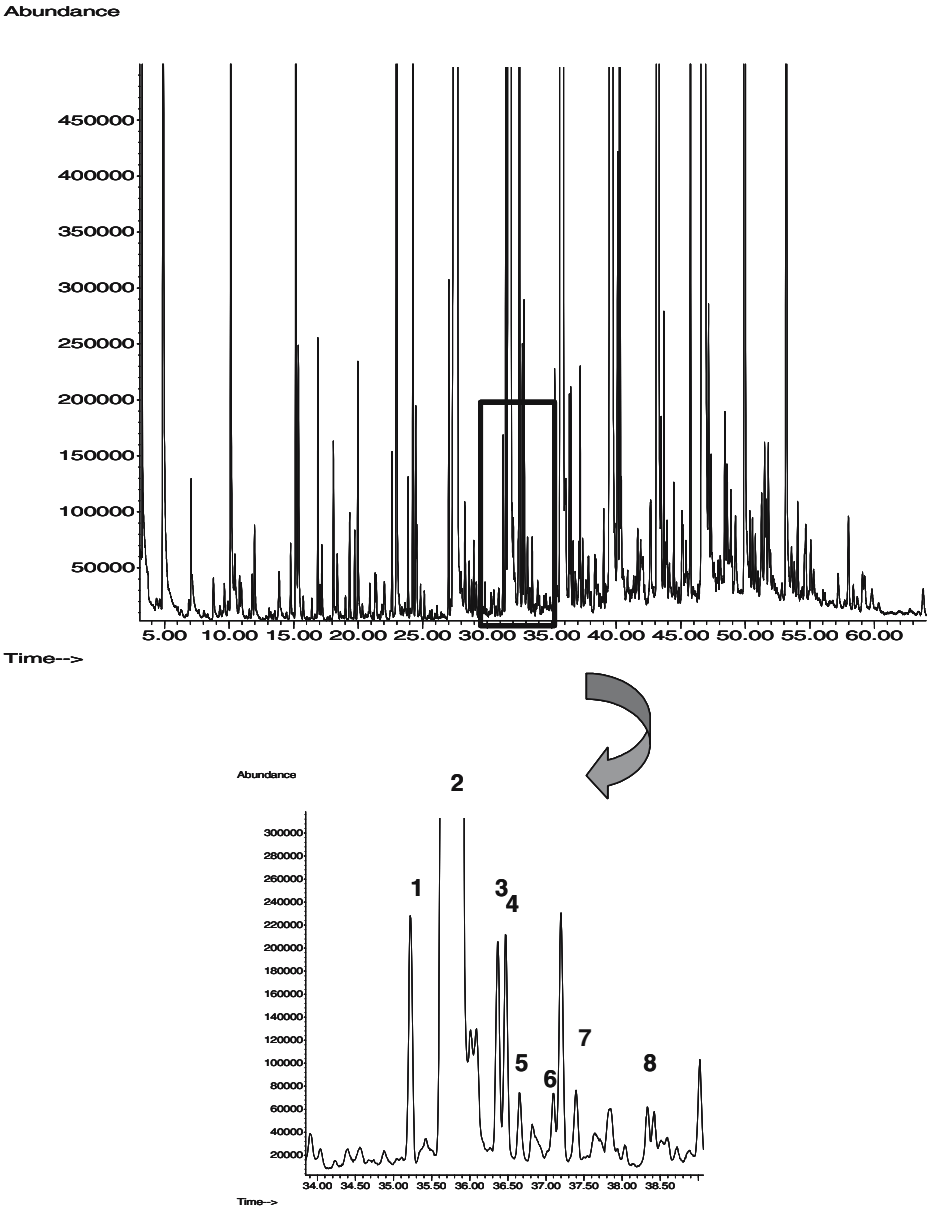

Fig. 1 A representative GC–MS TIC (total ion chromatogram) of the volatile compound profile extracted from preen oil. The enlargement of the framed area shows complexity of the profile leading to a partial co-elution of compounds. Numbers indicate the compounds 1: a methyl carboxylic acid, 2: 1-tridecanol, 3: 1-hexadecene, 4: 2-tetradecanone, 5: hexadecane; 6: 1-tetradecanol, 7: benzophenone, 8: a branched hydrocarbon

In addition to the TIC profiles, ions *m/z* 55, 58, and 60 were chosen to monitor peak areas of alcohols and aldehydes (*m/z* 55), a series of methylketones (*m/z* 58), and fatty acids (*m/z* 60). In Fig. 2, we demonstrate typical simplified profiles for *m/z* 55 and *m/z* 60. Six females and 6 males were investigated during both breeding and nonbreeding conditions

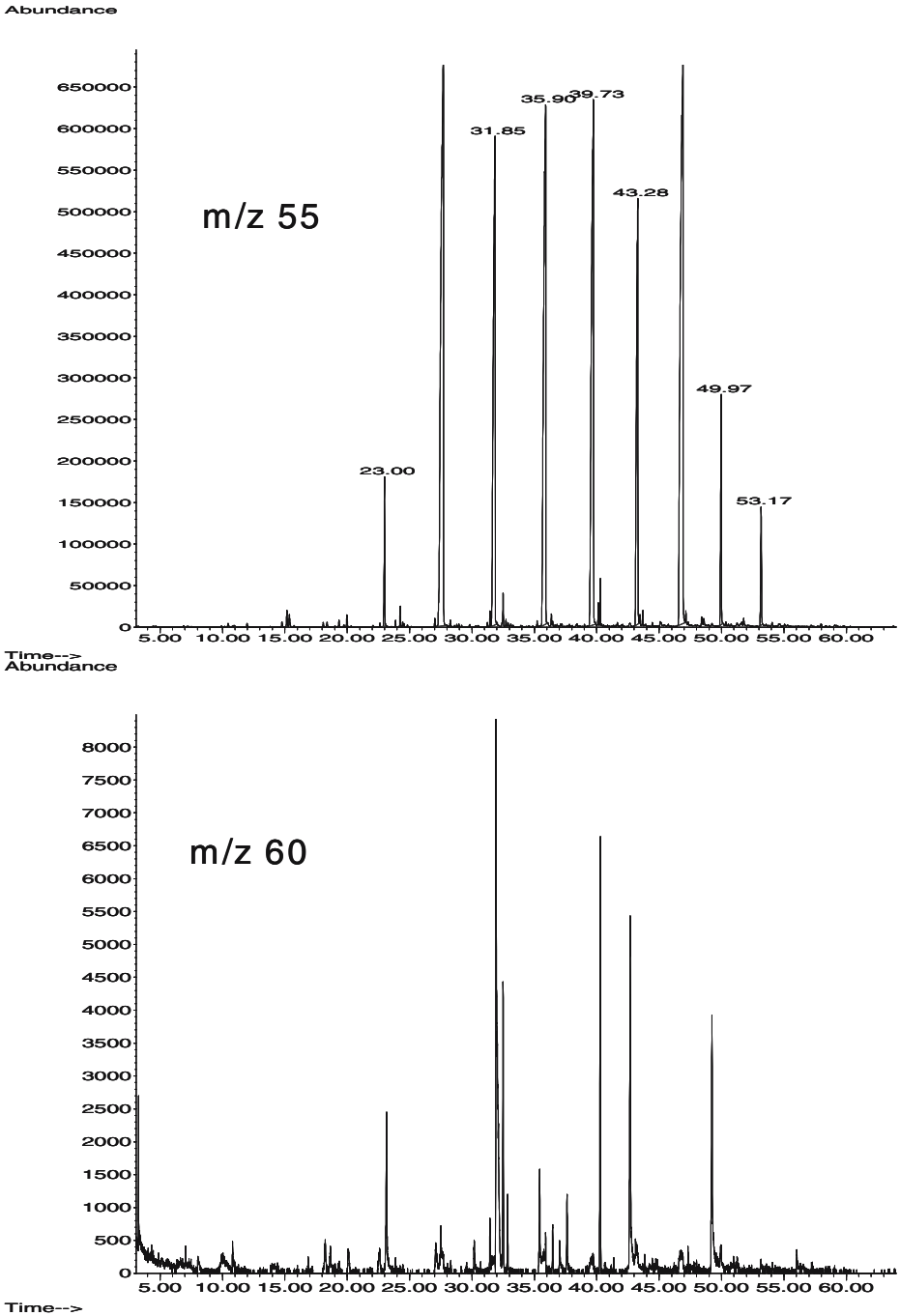

Fig. 2 Simplified selected-ion chromatograms (SICs) for extracted ions at m/z 55 and m/z 60 in a representative preen oil sample

through qualitative and quantitative comparisons. Table 1 lists the identified compounds together with their retention times and statistical significance classified by both season and sex. The compounds found prominently in preen oil samples included a homologous series of linear alcohols (C_9–C_{18}) as the major components in all samples (peak areas 100–1,000

Table 1 Compounds used for quantitative comparisons between breeding and nonbreeding conditions

	Compound	R_t (min)	TIC or Ion m/z	Female *P**	Male *P**
1	Octanal	11.05	55		
2	p-Cymene	11.98	TIC		
3	2-Ethyl-1-hexanol	12.00	TIC		
4	Undecane	15.28	TIC		
5	Nonanal	15.47	55		
6	1-Nonanol	18.62	55		
7	Decanal	20.17	55		
8	Nonanoic acid	22.90	60		
9	1-Decanol	23.20	55	0.01	0.004
10	Tridecane	24.39	TIC		
11	2-Undecanone	24.04	58		
12	Undecanal	24.66	TIC		
13	Decanoic acid	27.10	60		
14	1-Undecanol	27.87	55	0.01	0.002
15	Geranylacetone	30.55	TIC		
	Internal standard (added)	31.44	113		
16	1-Dodecanol	32.00	55	0.004	0.002
17	2-Tridecanone	32.69	58		0.002
18	Dodecanoic acid	35.16	60	0.028	
19	1-Tridecanol	36.00	55	0.16	0.002
20	2-Tetradecanone	36.30	58		0.002
21	1-Tetradecanol	39.82	55	0.028	0.002
22	2-Pentadecanone	40.49	58		0.004
23	Heptadecane	40.58	TIC		
24	3-Methyltridecanoic acid	40.79	TIC		
25	Tetradecanoic acid	42.73	60		0.044
26	1-Pentadecanol	43.44	55	0.01	0.002
27	A C18 alkene	43.63	TIC		
28	2-Hexadecanone	43.72	58		
29	1-Octadecene	43.87	TIC		
30	3-Methyltetradecanoic acid	44.34	TIC		
31	Hexadecanal	44.65	TIC		
32	An alkene	45.39	TIC		
33	1-Hexadecanol	46.95	55	0.016	0.002
34	2-Heptadecanone	47.15	58		
35	3-Methylpentadecanoic acid	47.65	TIC		
36	A C20 alkene	48.77	TIC		
37	Hexadecanoic acid	49.15	60	0.044	0.010
38	1-Heptadecanol	50.22	55		0.002
39	1-Octadecanol	53.50	55	0.044	0.002

*P**: Mann–Whitney *U* test (two-tailed) for significant seasonal differences for female (N=6) and male (N=6) matched pairs

times larger than minor compounds). Additionally, the series of aldehydes, acids, and methylketones were included in such volatile profiles. Among the minor components, some branched acids and saturated and unsaturated hydrocarbons were notably detected (see also Fig. 1).

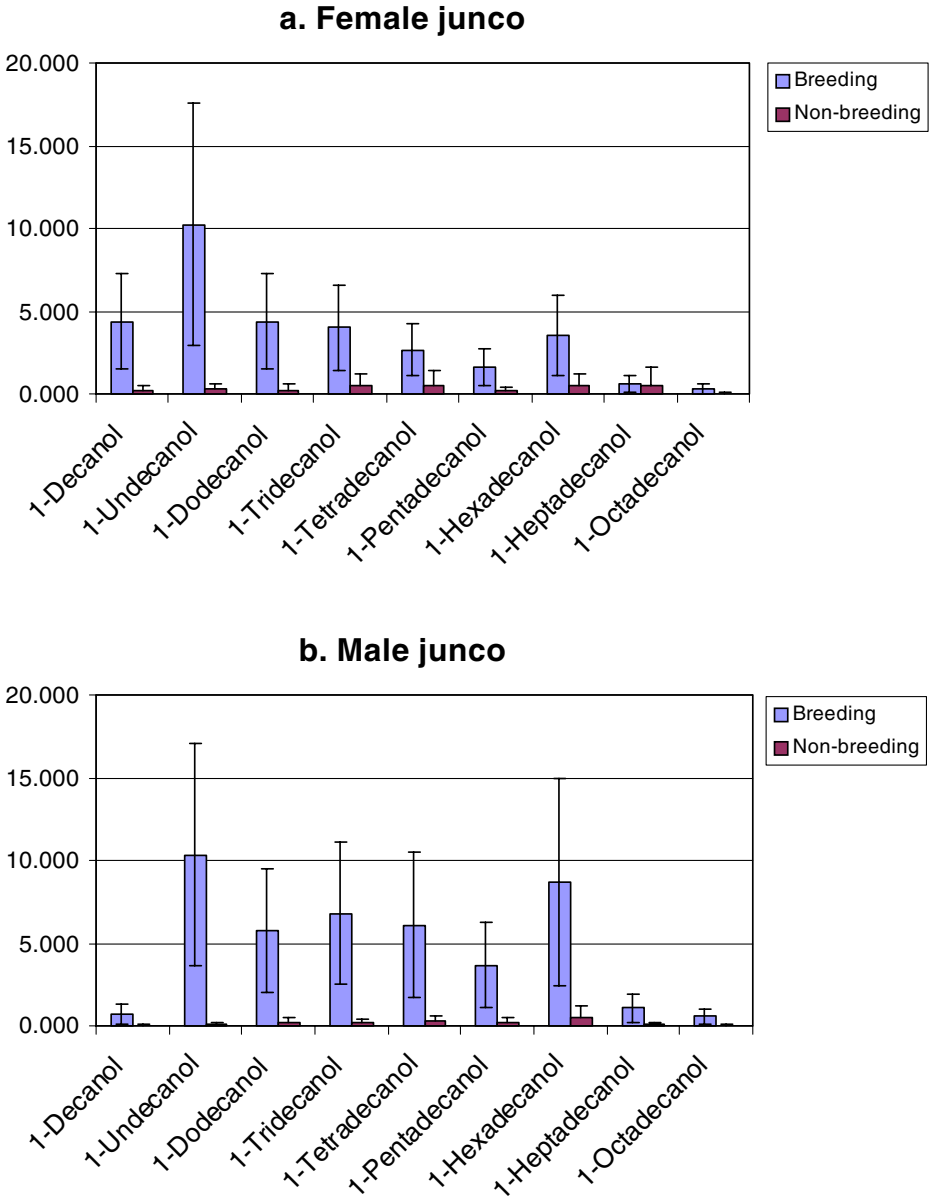

Fig. 3 (a) Averages of the normalized peak areas of C_{10}–C_{18} alcohols in female junco preen oil samples (N=6) in breeding and nonbreeding conditions. Error bars indicate individual variation (standard deviation, SD). (b) Averages of the normalized peak areas of C_{10}–C_{18} alcohols in male junco preen oil samples (N=6) in breeding and nonbreeding conditions. Error bars indicate individual variations (standard deviation, SD)

Linear Alcohols The highest levels of *n*-alkanols were detected as C_{10}–C_{18} (from decanol to octadecanol). Also, 1-nonanol, was detected at lower concentrations. Using the extracted ion profiles for *m/z* 55 (see Fig. 2), the peak areas of alcohols were measured and normalized with the peak area of the internal standard (*m/z* 113). In Fig. 3, we show the plotted normalized peak areas for the major alcohols of female and male juncos, respectively. In the breeding condition, as compared to the nonbreeding condition, both junco sexes showed elevated levels of these alcohols, although their individual variations appeared relatively large, as indicated by the error bars in Fig. 3.

Except for 1-heptanol in the female group, all *n*-alcohols, from 1-decanol to 1-octadecanol, showed statistically significant seasonal increases ($P<0.05$) in both males and females under long days. As seen in Table 1, based on their smaller *P* values, the differences tended to be larger in males than females. The compound 1-decanol was an exception in that it appeared more elevated in breeding females than in breeding males, but the sex difference was only marginally significant ($P<0.052$). During the nonbreeding season, concentrations of the linear alcohols did not differ between females and males.

Linear *n*-alcohols, such as those seen here as the major volatile components in the preen oil of the dark-eyed junco, have been reported as effective natural antimicrobial and antifungal substances for a wide spectrum of gram-positive bacteria, molds, and yeasts (Kubo et al., 1993, 1995, 2003). Indeed, these alcohols could have multiple functions during a junco breeding season as antimicrobial and antifungal agents. Juncos are high-latitude and high-altitude birds, and during winter their environments tend to be cold enough to suppress growth of both bacteria and fungi. However, warmer temperatures increase microbial growth, and alcohols may be a response to increased exposure, serving to promote healthy feathers and conditions at the nest.

In addition, plant leaves typically contain linear alcohols (Vioque and Kolattukudy, 1997). Increased levels of *n*-alcohols in preen oil during summer may help both female and

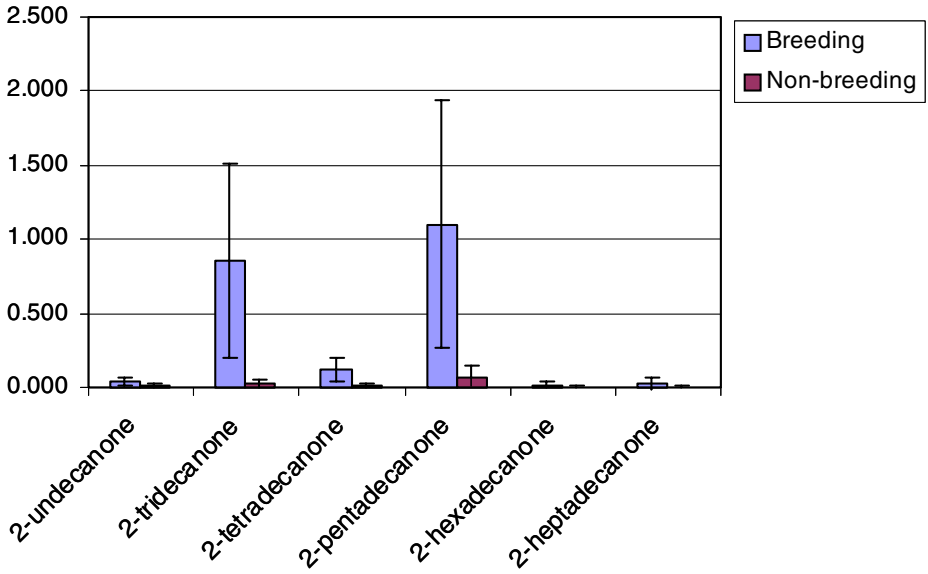

Fig. 4 Averages of the normalized peak areas of methylketones in male junco preen oil samples ($N=6$) in breeding and nonbreeding conditions. Error bars indicate individual variations (standard deviation, SD)

male juncos to blend their odors with the background odors of the plants in their environment (odor blending), for example, when the female with incubating eggs in a ground nest or the male is approaching the nest with food. Junco's eggs and young are a common prey of mammalian predators such as mice, chipmunks, and raccoons, so odor camouflage could provide a significant advantage (Nolan et al., 2002; Clotfelter et al., 2004).

Linear Methylketones A pattern of linear methylketones was seen in all preen gland samples (both male and female juncos). Comparing their peak areas to those of *n*-alkanols, methylketones were minor components. The peak areas for these compounds were measured using the extracted ion at *m/z* 58.

2-Tridecanone and 2-pentadecanone levels were higher than the remaining 2-ketones for the breeding male juncos, as shown in Fig. 4. A statistically significant increase (compared to nonbreeding season), was found for 2-tridecanone ($P<0.002$), 2-tetradecanone ($P<0.002$), and 2-pentadecanone ($P<0.004$) (see also Table 1). Female juncos showed an increase during the breeding season for 2-tridecanone and 2-pentadecanone levels, although these levels were somewhat lower than in males, and the changes were only close to statistical significance as a result of the large individual variations ($P<0.051$). A comparison between the male and female levels of 2-ketones showed only slight tendency for higher 2-pentadecanone levels in breeding males vs. breeding females ($P<0.186$).

Methylketones are also commonly found components in mammalian epidermis, including human skin (Penn et al., 2006). In birds, similarly to *n*-alcohols, methylketones may be promoting healthy feather conditions by acting as chemical defense compounds. Methylketone activity has previously been shown to provide plants with protection from herbivores and fungal pathogens, having been reported as a chemical defense compound in tree bark (McDowell et al., 1988), and as protection against the spider mite in wild and cultivated tomato plants (Chatzivasileiadis et al., 1999). Perhaps they provide similar protection for feathers.

Fatty Acids Preen samples contained linear and branched carboxylic acids at relatively low levels. The linear acid concentrations were higher than for the branched acids. The linear-

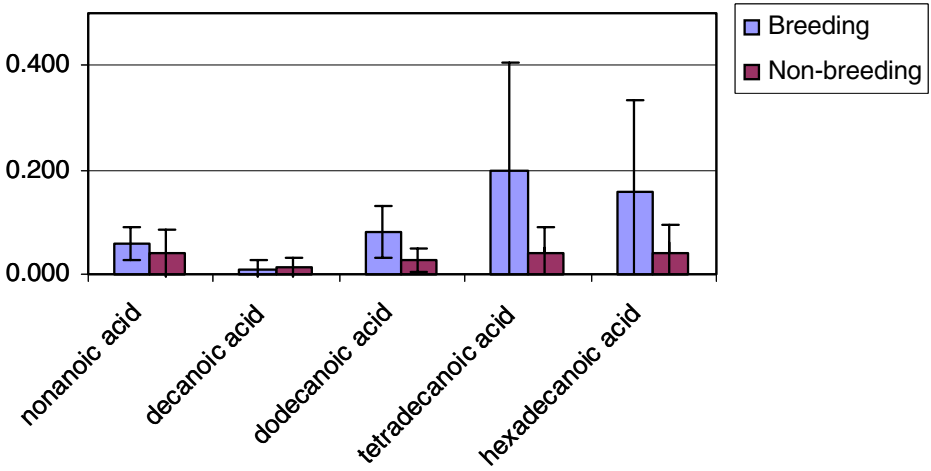

Fig. 5 Averages of the normalized peak areas of linear acids (C_9, C_{10}, C_{12}, C_{14}, C_{16}) in female junco preen oil samples ($N=6$) in breeding and nonbreeding conditions. Error bars indicate individual variations (standard deviation, SD)

acid peak areas were measured by using the extracted ion at *m/z* 60. Individual variation within all acid concentrations in the samples was quite large, as shown in Fig. 5, for the female juncos. Moreover, for this class of compounds, the differences between the breeding and nonbreeding conditions were not clear. However, in both female and male junco samples, tetradecanoic and hexadecanoic acid (even number fatty acids) levels were elevated more than for similar constituents (shown in Fig. 5) in the breeding condition. In both sexes, a statistically significant increase during breeding was found only for hexadecanoic acid ($P<0.044$ for females and $P<0.010$ for males). Additionally, the seasonal increase in dodecanoic acid was significant: for females, dodecanoic acid ($P<0.028$) and for males tetradecanoic acid ($P<0.044$). However, the acid concentrations did not differ statistically between females and males during the seasons.

Branched fatty acids were detected at lower levels than linear fatty acids, but also with large individual variations (data not shown). Their levels did not differ significantly by season in either male or female juncos.

Human sebum and circulating lipids contain a large number of different linear and branched fatty acids. It was suggested long ago that the ratios of the acidic constituents in mammalian skin lipids may carry an olfactory signature of an individual (Nicolaides, 1974). Interestingly, mammalian wax monoester biosynthesis involves fatty acids as starting materials, followed by reduction to linear alcohols (fatty alcohols) by a specific reductase enzyme. A wax synthase enzyme converts fatty alcohols finally to wax monoesters (Cheng and Russell, 2004a, b). Similarly, wax ester synthesis via linear alcohols was suggested for algae even earlier (Kolattukudy, 1970).

In our current study, the levels of nonvolatile wax monoesters were not of a direct interest. Whether their levels vary seasonally in the preen oil is not currently known. However, although fatty acid compositions were variable among the individuals across the seasons, the levels of linear alcohols (possibly fatty acid reduction products) increased dramatically. Accordingly, one might postulate that the wax ester synthesis route may slow down during the breeding season, yielding more *n*-alcohols as fatty acid reduction products.

One of the more interesting implications of volatile preen gland secretions is that the odors they create could serve to signal identity, condition, relatedness, or other information about an individual. This information might be utilized by conspecifics to make an assessment of the signaler. Compounds with large individual variation may be particular candidates as signals. The MHC gene products, for example, have been hypothesized both to facilitate immune responses (Apanius et al., 1997, Penn and Potts, 1999) and to produce recognizable odors (Penn and Potts, 1998, Spehr et al., 2006). Furthermore, some studies have speculated that these odors might be used to promote adaptive mate choice, as they allow individuals to assess their relatedness and regulate their degree of inbreeding (Potts and Wakeland, 1990; Brown and Eklund, 1994; Penn and Potts, 1999).

Interestingly, *CD1* genes (evolutionary predecessors of the MHC genes) which have been found in humans, have also been identified in birds. Correspondingly, these genes code for proteins that present antigens such as fatty acids and glycolipids to T cells, rather than the more typical presentation of peptides (Miller et al., 2005; Salomonsen et al., 2005). These genes might also play a role in regulating similar fatty acid compositions as found in preen gland secretions (Porcelli and Modlin, 1999). Enhanced detection of variation in the *MHC* or *CD1* genes might also be useful for dark-eyed juncos, particularly for those that breed in the Appalachian Mountains where natal philopatry is high (Nolan et al., 2002) and thus the potential for inbreeding is also high. Individuals able to detect variation at the MHC and, possibly CD1 loci, could potentially benefit from inbreeding avoidance and increased offspring fitness by maintaining immunogenetically important MHC or CD1 heterozygosity.

Comparisons of the other compounds, which appeared at low levels (e.g., *p*-cymene, saturated and unsaturated hydrocarbons, and some aldehydes) did not result in clear differences by sex or between the breeding and nonbreeding conditions (data not shown).

Sulfur Compound Profiles Several individuals from each group of breeding and nonbreeding males and females were qualitatively screened using the ultrasensitive sulfur compound detection (GC-AED). The sorptive stir bar extraction method has been previously shown to recover efficiently sulfur compounds at picogram quantities from biological samples (Soini et al., 2005). In this study, the breeding males consistently showed a higher number of low (picogram level) sulfur compounds than nonbreeding males and females (breeding or nonbreeding). No identifications could be made because of the low levels detected in the sulfur detection mode (data not shown).

Volatile Compound Profiles on the Feather Surface Among the volatile compounds collected from the wing surface were several compounds, including *n*-alcohols, which were obviously shown as originating from the preen oil (see Fig. 6). Additionally, numerous compounds not found previously in preen oil were detected on the feather surface. These compounds may be originating from the bird epidermal lipids (Menon and Menon, 2000), their surface-catalyzed conversions, or environmental sources. A large porous surface area on feathers is known to be efficient in enriching airborne particulates, including metals. Several studies have utilized bird feather surface for a biological monitoring of environmental heavy metal pollutants (for review see, Burger, 1994; Veerle et al., 2004).

In summary, the volatile compounds found in *J. hyemalis* preen gland secretions were found to have increased during the breeding season in both sexes, and the seasonal variations may reflect a number of possible functions for these components including defense against bacteria and fungi, odor camouflage in relation to nest predators, or information to

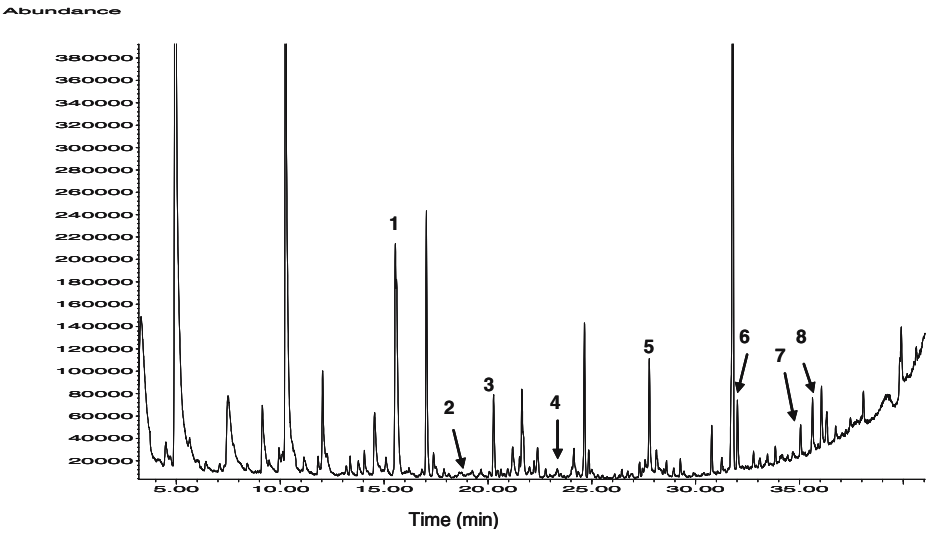

Fig. 6 A volatile compound GC–MS TIC profile collected directly from the surface of the male junco wing feather. Compounds, which were also found in the preen oil, are marked by numbers 1: nonanal, 2: 1-nonanol, 3: decanal, 4: 1-decanol, 5: 1-undecanol, 6: 1-dodecanol, 7: dodecanoic acid, 8: 1-tridecanol

be communicated to conspecifics, such as chemosignals about condition (appearance) or genotype (degree of relatedness). A large individual variation in the volatile compound levels could even indicate their regulation by *MHC* or *CD1* genes. More experiments are needed to test possible functions of the linear alcohols, methylketones, and linear carboxylic acids whose concentration increased during the breeding. Such experiments will require a carefully controlled design of behavioral and hormonal conditions.

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