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Songbird chemosignals: volatile compounds in preen gland secretions vary among individuals, sexes, and populations

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Chemical signaling has been documented in many animals, but its potential importance in avian species, particularly songbirds, has received far less attention. We tested whether volatile compounds in the preen oil of a songbird (*Junco hyemalis*) contain reliable information about individual identity, sex, or population of origin by repeated sampling from captive male and female juncos originating from 2 recently diverged junco populations in southern California. One of the populations recently colonized an urban environment; the other resides in a species-typical montane environment. The birds were field-caught as juveniles, housed under identical conditions, and fed the same diet for 10 months prior to sampling. We used capillary gas chromatography–mass spectrometry to quantify the relative abundance of 19 volatile compounds previously shown to vary seasonally in this species. We found individual repeatability as well as significant sex and population differences in volatile profiles. The persistence of population differences in a common environment suggests that preen oil chemistry likely has a genetic basis and may thus evolve rapidly in response to environmental change. These finding suggest that songbird preen oil odors have the potential to function as chemosignals associated with mate recognition or reproductive isolation. *Key words:* birds, chemical communication, *Junco hyemalis*, olfaction, pheromones. *[Behav Ecol 21:608–614 (2010)]*

Recent studies indicate that olfactory communication may play an important role in reproductive behavior in birds; most work to date has focused on seabirds of the order Procellariiformes (Bonadonna and Nevitt 2004; Hagelin 2007) and fowl in the order Galliformes (Taziaux et al. 2008; Hirao et al. 2009). These olfactory signals may be transmitted via compounds present in preen oil secreted from the uropygial gland (Hirao et al. 2009). Birds spread this oil onto their plumage where it has been thought to function primarily to protect the feathers from environmental degradation, enhance their insulative capacity, and to ward off ectoparasites such as feather lice (Jacob and Ziswiler 1982; but see Moyer et al. 2003). Preen oil is now known to contain volatile compounds that contribute to an odor (Haribal et al. 2005, 2009; Douglas 2006; Soini et al. 2007). In some species, the odor from this oil may serve to repel predators (Burger et al. 2004; Douglas et al. 2004; Hagelin and Jones 2007). Behavioral studies suggest odor may also be important in intraspecific communication in seabirds (Hagelin et al. 2003; Bonadonna and Nevitt 2004; Hagelin 2007), chickens (Hirao et al. 2009), and even in passerines (Whittaker et al. 2009). Concentrations of volatile compounds in preen oil vary seasonally, perhaps in relation to hormonal status (Piersma et al. 1999; Soini et al. 2007; Douglas et al. 2008), and the compounds themselves differ among species (Haribal et al. 2005, 2009). At least one study has found that seabird odor is variable among individuals and that individual odor is repeatable (Bonadonna et al. 2007). In songbirds, the use of chemical signals is virtually unexplored, although at least one study suggests that songbirds are capable of distinguishing between preen oil odors from conspecifics and heterospecifics (Whittaker et al. 2009). The order Passeriformes is the most speciose group of birds with over 6000 species and displays enormous diversity in visual and auditory signals (Gill 2006), yet only a few studies have addressed interspecific variation in the volatile compounds found in preen oil (Haribal et al. 2005, 2009) or seasonal variation (Soini et al. 2007). No prior study has examined either individual repeatability or intraspecific variation in songbird preen gland secretions.

We examined the volatile content of preen oil from a well-studied songbird, the dark-eyed junco (*Junco hyemalis*), to evaluate its potential utility as a chemosignal. Volatile compounds in junco preen oil have been previously described and are known to vary with season (day length) and to increase during the breeding season (Soini et al. 2007), suggesting a potential role in reproductive behavior. We asked whether volatile compounds in the preen oil of juncos would meet several of the prescribed criteria for compounds that might serve as reproductive chemosignals (Johansson and Jones 2007); specifically, we tested the hypotheses that they would: 1) be repeatable within an individual (e.g., individuals should have distinct chemical "signatures"), 2) differ consistently between males and females, and 3) differ among geographically disjunct populations of the same species. We compared preen oil volatile composition between dark-eyed juncos that were originally captured from 2 recently diverged populations in San Diego County, CA, following one population's unique colonization of a novel urban environment. These birds were captured as young juveniles and held in a captive "common garden"

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experiment under identical conditions for 10 months prior to sampling. Details of the study system are described below.

MATERIALS AND METHODS

Study system and sample collection

A small number of breeding pairs of Oregon juncos (*J. hyemalis thurberi*) colonized the University of California, San Diego (UCSD) campus in the early 1980s; since that time this population has remained isolated geographically and genetically, about 70 km from the nearest breeding population with low levels of immigration (Rasner et al. 2004; Yeh and Price 2004). Several morphological and behavioral changes have occurred in this short period of time, including cessation of migration, reduced wing length, reduction of a sexually selected plumage characteristic, shifts in reproductive behavior including a longer breeding season, reduced territorial aggression, and increased exploratory boldness compared with juncos in the presumptive ancestral range (Rasner et al. 2004; Yeh 2004; Yeh and Price 2004; Newman et al. 2006; Yeh et al. 2007; Price et al. 2008; Atwell JW, unpublished data). Data from common garden experiments suggest that changes in plumage traits and exploratory boldness may have a genetic basis (Yeh 2004; Atwell JW, unpublished data).

Juncos in this study were originally captured from the UCSD campus (lat 32°40′N, long 117°10′W; elevation 30 m) and the Laguna Mountain Recreation Area in the Cleveland National Forest (lat 32°52′N, long 116°25′W; elevation 1700 m). These birds were captured as juveniles (recognizable by a distinct plumage) soon after they became independent (30–40 days posthatch) in June and July 2007, using the same capture methods (mist nets and walk-in traps) for both populations. After capture, juveniles were housed in flocks in temporary outdoor aviaries in suburban San Diego, CA until mid-July 2007, then shipped via air cargo to the Kent Farm Bird Observatory (KFBO) indoor aviaries at Indiana University in Bloomington, IN. At KFBO, the birds were segregated by population into 2 large identical windowless aviary rooms (6.4 m L × 3.2 m W × 2.4 m H) with equivalent densities (~1 bird/m^2). Within each room, birds were housed in cages in mixed-sex pairs. Birds were segregated by population but otherwise held in identical conditions. The light schedule was set to match the photoperiodic schedule of the native breeding latitude of the 2 populations (which is the same). Temperature was maintained at 60–65 °F. Birds were given ad libitum access to water, seed, fruit, and mealworms in each cage. All aviary rooms had equivalent exposure to human researchers and animal care staff.

Five times during a 2-week period (at 2- to 3-day intervals) in June 2008, we repeatedly sampled preen oil from 26 captive juncos of both sexes from the 2 populations: from Laguna Mountain, 8 females and 6 males and from UCSD, 6 females and 6 males. Birds were sampled in a random order on each sampling day. In order to minimize handling stress during preen oil collection, we captured individuals from cages by darkening the lights in the room and quickly catching the target individual by hand and then keeping handling times to under 5 min. All birds were subjected to similar handling procedures. We collected preen oil by gently pressing a 100-µl glass micropipette tube (Drummond Scientific Company, Broomall, PA) against the uropygial gland and rubbing until a small amount (1–3 mg) of preen oil was secreted. Once collected, preen oil samples were stored at −20 °C until analyzed using gas chromatography–mass spectrometry (GC-MS) and gas chromatography–atomic emission detection (GC-AED).

Sample preparation

Using a Teflon plunger, we pushed a thawed preen oil sample into a cleaned 20-ml glass vial and added 2.0 ml of water (high-purity OmniSolv, EMD Chemicals, Inc., Gibbstown, NJ), 100 mg of ammonium sulfate (99.99 + % from Sigma-Aldrich, St Louis, MO), and an internal standard (8 ng of 7-tridecanone, Sigma-Aldrich) dissolved in 5 µl of methanol (Baker Analyzed, Mallinckrodt Baker, Inc., Phillipsburg, NJ). Volatile compounds were extracted with the Twister stir bar (10 × 0.5 mm polydimethylsiloxane) for 60 min (Gerstel GmbH, Mülheim an der Ruhr, Germany). After extraction, the stir bar was rinsed with high-purity OmniSolv water, dried with a paper tissue, and placed in the thermal desorption autosampler tube.

GC-MS

Quantitative analysis was performed using the Agilent 6890N gas chromatograph connected to a 5973i MSD mass spectrometer (Agilent Technologies, Inc., Wilmington, DE) with the thermal desorption autosampler and cooled injection system (TDSA-CIS 4 from Gerstel). Positive electron ionization mode at 70 eV was used with a scanning rate of 2.47 scans/s over the mass range of 41–350 amu. The mass spectrometric detector (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, inner diameter [i.d.], 0.25-µm film thickness) from Agilent (J&W Scientific, Folsom, CA). 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 and the 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/s to 270° C, with a hold time of 12 min. CIS inlet was operated in the solvent vent mode, a vent pressure of 9.1 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: 12 min). The carrier gas head pressure was 9.1 psi (flow rate, 1.1 ml/min at the constant flow mode).

GC–atomic emission spectrometry

Qualitative element-selective compound profiling was performed using a GC 6890 instrument equipped with an atomic emission detection system (AED, model G2350A) from Agilent Technologies and a thermal desorption autosampler-cooled injection system (TDSA-CIS-4 from Gerstel). The separation capillary was 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. Temperature of the transfer line was 280 °C. The emission lines for carbon (193 nm), sulfur (181 nm), and nitrogen (174 nm) were monitored during the atomic plasma emission detection.

Quantitative comparisons

Among approximately 100 compounds detected in the preen oil, about 40 components of chromatographic profiles were tentatively identified. All major compounds were positively identified by comparison with standard substances of known mass spectra and retention times. Peak areas of the identified compounds were used for quantitative comparisons among the groups. Peak areas were integrated either from the total ion current (TIC) profiles or from the postrun single 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 postrun 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. Relative standard deviation (a measure of reproducibility) of the internal standard peak area was 13% ($n = 12$).

Qualitative comparisons

The GC-AED system provides about 100 times more sensitivity for sulfur-containing organic compounds at the sulfur emission line (181 nm) than GC-MS measurements. Despite this ultrahigh detection sensitivity, no consistently appearing sulfur-containing compounds were detected in the junco preen oil samples.

Statistics

We focused on 19 volatile compounds, quantified by GC-MS, that were previously found to vary seasonally in juncos and to increase during the breeding season (Soini et al. 2007; Soini HA and Whittaker DJ et al., unpublished data), suggesting their possible role in reproductive behavior. We examined individual "volatile profiles" of compounds by testing for repeatability and individual differences in the relative proportions of each compound (Svensson et al. 1997; Miklas et al. 2000). GC-MS peak areas were measured, and for each volatile compound, the observed GC-MS peak area was converted to a percentage of the total observed peaks. Because the proportion data are not normally distributed, we then logit transformed the data by taking the natural logarithm of $(p/(1 - p))$, where p is the proportion (Armitage and Berry 1994).

We calculated repeatability (r) of the relative proportions of individual volatile compounds in repeated preen oil samples from the same individual using the following formula:

$$R = [(M_{SA} - M_{SW})/n_0]/[M_{SW} + [(M_{SA} - M_{SW})/n_0]],$$

where MS_A is the among-groups variance component (variation among individuals), MS_W is the within-groups variance component (variation within individuals), and n_0 is the sample size (Lessells and Boag 1987).

After determining that relative measurements of volatile compounds were highly repeatable for each individual, we averaged the 5 measurements to obtain a single measurement for each volatile compound for each individual to avoid pseudoreplication before proceeding. We conducted a principal components analysis (PCA) (SPSS 16.0) using 17 of the identified volatile compounds (2 compounds, nonanoic acid, and decanoic acid, were below detectable levels in several individuals and were excluded from the analysis) and rotated the component matrix to maximize variance (varimax rotation). We then tested for differences between groups by conducting a multivariate analysis of variance (MANOVA) using sex and population as fixed factors and the synthetic variables generated from the PCA as the dependent variables.

RESULTS

The most common compounds in junco preen oil included linear 1-alkanols, methyl ketones, and carboxylic acids (Supplementary Table S1). The most concentrated volatile compounds, which combined made up ~75–85% of an individual's overall volatile profile, were the six 1-alkanols C_{11}–C_{16}; the next most concentrated compounds were the 1-alkanol C_{10}, the 2 methyl ketones 2-tridecanone and 2-pentadecanone, and a carboxylic acid, tetradecanoic acid.

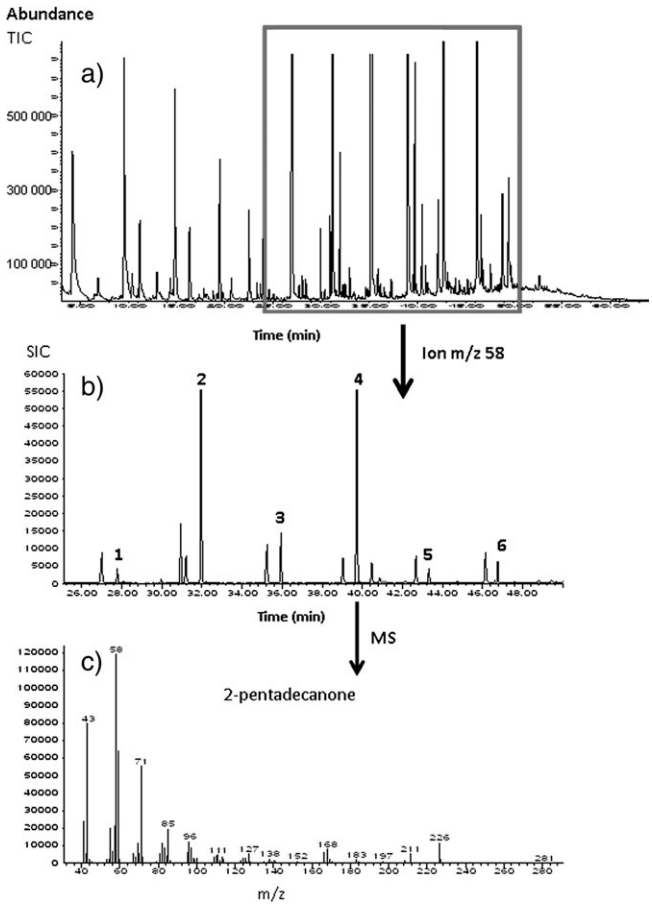

Figure 1
(A) A TIC profile obtained from male UCSD junco preen oil, (B) a corresponding postrun single ion current (SIC) profile of methyl ketones (m/z 58) from the time range 25–50 min (1: 2-dodecanone, 2: 2-tridecanone, 3: 2-tetradecanone, 4: 2-pentadecanone, 5: 2-hexadecanone, 5: 2-heptadecanone), and (C) the mass spectrum of 2-pentadecanone from the SIC profile (B).

Figure 1 illustrates a TIC profile obtained from preen oil of a male UCSD junco, a corresponding postrun single ion current (SIC) for methyl ketones (m/z 58) and the mass spectrum of 2-pentadecanone.

Repeatability

Relative concentrations of all compounds except one (nonanoic acid) differed significantly among individuals (1-way ANOVA, $P < 0.01$, Table 1). Repeatability was above 0.5 for 14 of the 19 compounds and was generally high, ranging from 0.08 to 0.91. For the 6 main linear alcohols that made up 75–85% of an individual junco's volatile compound profile, repeatability ranged from 0.70 to 0.87 (Table 1).

PCA

The PCA resulted in 5 principal components with Eigenvalues above 1; these principal components explained 38%, 20%, 15%, 9%, and 6% of the variance, respectively. The rotated component matrix is shown in Table 2. The first principal component is associated positively with the 5 methyl ketones and negatively with the 1-alkanols, 1-decanol, and 1-undecanol. PC2 is associated positively with 1-alkanols C_{12}–C_{15}. The third principal component is associated positively with carboxylic acids dodecanoic acid, tetradecanoic acid, and hexadecanoic acid. PC4 is

Table 1
Repeatability estimates for volatile compounds in junco preen oil (n = 26 individuals)

Compound	F	p	r
1-Nonanol	2.354	0.002	0.21
1-Decanol	12.727	<0.001	0.70
1-Undecanol	34.632	<0.001	0.87
1-Dodecanol	13.182	<0.001	0.71
1-Tridecanol	24.791	<0.001	0.82
1-Tetradecanol	30.758	<0.001	0.86
1-Pentadecanol	29.540	<0.001	0.85
1-Hexadecanol	12.796	<0.001	0.70
1-Heptadecanol	10.874	<0.001	0.66
2-Undecanone	12.548	<0.001	0.70
2-Dodecanone	5.928	<0.001	0.50
2-Tridecanone	52.643	<0.001	0.91
2-Tetradecanone	20.233	<0.001	0.79
2-Pentadecanone	37.161	<0.001	0.88
Nonanoic acid	1.420	0.159	0.08
Decanoic acid	2.809	0.005	0.27
Dodecanoic acid	5.923	<0.001	0.50
Tetradecanoic acid	3.860	<0.001	0.36
Hexadecanoic acid	3.760	<0.001	0.36

associated positively with 1-hexadecanol and 1-heptadecanol; PC5 is associated with 1-nonanol.

MANOVA

The principal components of volatile profiles differed significantly by sex (MANOVA, $F = 27.627$, $P < 0.001$) and population ($F = 3.518$, $P = 0.022$). The interaction between sex and population was also significant ($F = 3.218$, $P = 0.030$), indicating that the effect of sex on volatile profiles differed by population. Specifically, the model revealed significant variability between both sexes and populations in the first principal component ($F = 32.272$, $P < 0.001$, Figure 2). The second principal component was not significantly predicted by the model ($F = 1.886$, $P = 0.161$). Principal component 3 was significant overall ($F = 4.591$, $P = 0.012$); however, when examining comparisons at the sex and population level, PC3 was not significantly affected by sex ($F = 1.898$, $P = 0.182$) and nearly so by population ($F = 3.632$, $P = 0.07$), but the interaction effect of sex and population was significant ($F = 8.270$, $P = 0.009$). Principal components 4 and 5 were not significantly affected by sex or population (PC4, $F = 0.323$, $P = 0.809$; PC5, $F = 1.290$, $P = 0.303$).

Table 2
Eigenvalues, percentage of variance explained, and rotated component matrix (varimax rotation) for the 5 principal components extracted from the relative proportions of volatile compounds measured using GC-MS

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	6.418	3.477	2.624	1.465	1.103
% Variance explained	37.751	20.453	15.436	8.618	6.489
1-Nonanol	0.019	−0.144	0.080	0.143	**0.854**
1-Decanol	**−0.708**	0.091	−0.254	−0.358	0.342
1-Undecanol	**−0.848**	−0.098	0.042	−0.389	0.042
1-Dodecanol	−0.343	**0.635**	−0.373	−0.323	0.109
1-Tridecanol	0.027	**0.902**	−0.194	−0.018	−0.189
1-Tetradecanol	−0.316	**0.857**	−0.125	0.235	−0.086
1-Pentadecanol	−0.035	**0.800**	−0.068	0.535	0.000
1-Hexadecanol	−0.211	0.321	−0.110	**0.885**	0.004
1-Heptadecanol	0.314	−0.048	0.150	**0.759**	0.421
2-Undecanone	**0.915**	0.102	0.113	−0.136	0.195
2-Dodecanone	**0.847**	−0.057	0.105	0.054	0.346
2-Tridecanone	**0.871**	−0.398	−0.009	−0.194	−0.099
2-Tetradecanone	**0.893**	−0.280	0.038	−0.005	0.041
2-Pentadecanone	**0.922**	−0.180	−0.071	−0.080	−0.154
Dodecanoic acid	−0.072	−0.170	**0.879**	−0.153	0.254
Tetradecanoic acid	0.108	−0.128	**0.961**	−0.065	−0.042
Hexadecanoic acid	0.066	−0.138	**0.912**	0.200	−0.028

Bold text indicates volatile compounds strongly associated with each principal component.

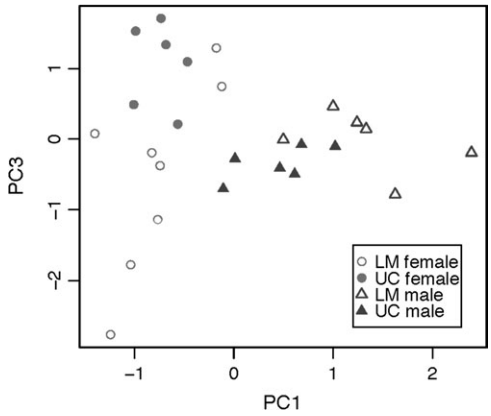

Figure 2
Principal component 1 and principal component 3 scores derived from relative proportions of volatile compounds. (See Table 2 for principal component loadings.)

DISCUSSION

We examined volatile compounds in the preen oil of dark-eyed juncos from 2 populations of the same subspecies living in a common environment and found high individual repeatability, significant differences between the sexes, and significant differences between populations. The differences between individuals and populations cannot be accounted for by differences in environment, diet, or stage of development as they were housed in identical captive conditions for 10 months and were of the same age. Because the differences between populations persisted in a common environment, we conclude that a genetic component to the differences is highly likely (Sun and Müller-Schwarze 1998; LeMaster and Mason 2003; Yeh 2004).

Individual repeatability

Relative proportions of volatile compound concentrations were highly repeatable within individuals. Because volatile chemosignals are produced metabolically, it has been suggested that they vary within an individual with reproductive condition and health and are not likely to transmit information about individual identity; in contrast, peptide chemosignals are genetically encoded and their information remains constant (Brennan and Zufall 2006; Touhara 2008). Although volatile chemosignals in juncos do appear to vary with reproductive status (Soini et al. 2007), the high repeatability suggests that they may also be capable of communicating individual identity. Although the time period covered by our study is only 2 weeks, our data suggest that day-to-day fluctuations in condition or behavior do not have a significant effect on volatile compound composition of songbird preen oil and also confirms the precision of our measurement methods. Other studies suggest that genotype likely

affects volatile chemosignals: volatile compounds on Antarctic prion feathers were highly repeatable among different years (Bonadonna et al. 2007), volatile profiles of ring-tailed lemurs were correlated with genetic diversity (Charpentier et al. 2008), volatile compounds in beavers were associated with family membership (Sun and Müller-Schwarze 1998), and volatile compounds in mouse urine reflect major histocompatibility complex (MHC) genotype (Novotny et al. 2007). Our results provide the first evidence that volatile compounds in songbird preen oil may be correlated with genotype.

Sex differences

Based on these data we can begin to understand sex differences in junco volatile profiles and identify potential sex-specific chemosignals. A "female-like" volatile profile has higher proportions of 1-undecanol, dodecanoic acid, tetradecanoic acid, and hexadecanoic acid. Notably, the female carboxylic acid blend favors the even-numbered carboxylic acids C-12, C-14, and C-16, mimicking the natural plant oil even-numbered fatty acid distribution (Brady et al. 1960). A "male-like" profile has higher proportions of the methyl ketones 2-undecanone through 2-pentadecanone; in particular, the relative proportions of 2-tridecanone and 2-pentadecanone are 2–4 times higher in males than in females. Such methyl ketones have been found as odoriferous compounds in insect gland secretions (reviewed in Forney and Markovetz 1971). They predominantly appear with odd-numbered carbon chains, which is traditionally thought to be due to β-oxidation of even-numbered carboxylic acids (Dakin 1908). Experimental work found that pancreatic lipase enzyme converted hexanoic, octanoic, and decanoic acids to 2-pentanone, 2-heptanone, and 2-nonanone, respectively (Pannell and Olson 1991). Dominant 2-tridecanone and 2-pentadecanone in male junco preen oil could therefore originate from tetradecanoic and hexadecanoic acids through enzymatic conversions. Further experiments are needed to verify if this biosynthetic pathway also occurs in junco preen oil. Many sex differences are mediated by androgens; further work is needed to determine whether sex-specific developmental hormones (e.g., testosterone) affect the observed sex differences in volatile compounds.

Population differences

Given the very recent divergence between the 2 populations of California juncos in other characters related to reproduction (life history, sexually selected plumage), it is reasonable to speculate that the population differences observed in both males and females are evidence of developing divergence in cues that might ultimately contribute to reproductive isolation between the populations. For example, in garter snakes, reproductive isolation exists between populations as a result of a change in the relative concentrations of components of the female sexual attractiveness pheromone in one population (LeMaster and Mason 2003). Several of the changes observed in the UCSD junco population, including reduced migratory activity, change in length of breeding season, and tameness, are characteristic of urban populations of other bird and mammal species (Luniak 2004; Partecke et al. 2004; Bonier et al. 2007; Partecke and Gwinner 2007) and may be adaptations to an urban environment. Local adaptation may be facilitated by reproductive isolation and avoidance of outbreeding; thus, we may expect to find evidence of reproductive isolation in signaling characters. Divergence between the UCSD and Laguna Mountain junco populations has been observed previously in visual signals: UCSD juncos have a reduced amount of white in the outer tail feathers ("tail-white," Yeh 2004). Multiple cues in different sensory modalities may communicate the same information, particularly about individual quality, and thus elicit a greater reaction, improve the receiver's detection of the signal, or stimulate choice by receivers with different preferences (Candolin 2003).

Alternatively, distance between individual volatile profiles may correlate with genetic distance, and the difference between the populations may simply reflect reduced relatedness between populations compared with that within populations (Sun and Müller-Schwarze 1998). The UCSD population has reduced genetic diversity at neutral microsatellite loci (Rasner et al. 2004); interestingly, male and female UCSD juncos displayed reduced variation in preen oil volatiles compared with Laguna Mountain juncos (Figure 2).

Although it is possible that microclimate differences in the aviary rooms may account for some of the variation between the populations, we consider this possibility unlikely, as every effort was made to provide the birds with identical conditions—including room size, cage size, light schedules, ambient temperatures, food supply, population density, and handling stress.

Comparison with other species

The most abundant volatile compounds in junco preen oil are 1-alkanols followed by linear and branched carboxylic acids, aldehydes, and saturated and unsaturated hydrocarbons; the minor compounds are methyl ketones (Soini et al. 2007). Some of the same 1-alkanols and linear carboxylic acids have been found in feathers of the Antarctic prion, a seabird, although many other compounds were present in the blend (Bonadonna et al. 2007). A diverse range of compounds has been reported in the preen oil tropical and temperate bird species by Haribal et al. (2009); some linear alcohols and carboxylic acids were found in some of the tropical birds but not in all the investigated species. These species differed in abundance, number of compounds, and combination of compounds (Haribal et al. 2009). Secretions from European woodhoopoes and green woodhoopoes contain a complex blend of volatile compounds, most of which are not shared by other bird species, and evidence suggests that these compounds are produced by symbiotic bacteria (Burger et al. 2004; Martín-Vivaldi et al. 2010). Our recent investigations into volatile compound composition of several passerine bird species confirm that a volatile compound blend consisting primarily of an abundant series of linear 1-alkanols appears to be specific to juncos (Soini HA and Whittaker DJ et al., unpublished data). Based on studies to date, there do not seem to be any volatile compounds that are common to the preen oil of all bird species or even of all passerines.

Preen oil as a chemosignal source?

Songbirds are well known to use visual and auditory cues to assess potential mates. Results of this study indicate that birds vary in chemical cues in ways that would allow them also to employ olfaction when discriminating among potential mates. Individuals may vary in their perceptual ability in different sensory modalities; this variation may be overcome by sending the same information via different cues (Candolin 2003; Hebets and Papaj 2004). Therefore, recent findings regarding avian olfactory abilities (reviewed in Hagelin and Jones 2007; Balthazart and Taziaux 2009) and signaling theory both predict that bird species' use of olfactory chemical signals could be widespread. However, few studies have examined the potential role of preen oil as a source of chemical signal in avian taxa and fewer still in

songbirds, despite their extraordinary diversity and sensory capabilities.

Chemical signals used in reproductive behavior may convey information about the sender that allows the receiver to recognize mates (species recognition, as well as the appropriate sex in the appropriate reproductive condition) and to evaluate potential mates (Johansson and Jones 2007). Signals involved in mate assessment are predicted to advertise individual identity and quality; such signals may also be useful in other forms of social interactions such as dominance hierarchies (Johansson and Jones 2007). Information contained in mate assessment signals may include current condition (health, parasite load) or genetic makeup, including genotype (such as MHC genotype), overall genetic variation, or genetic compatibility with the receiver (Penn 2002; Wyatt 2003; Johansson and Jones 2007). Chemosignals are often involved in premating reproductive isolation between species (LeMaster and Mason 2003; Johansson and Jones 2007; Smadja and Butlin 2009). Thus, chemosignals used in reproductive behavior should meet some or all the following criteria: they should 1) differ among species, to allow species recognition; 2) differ between the sexes, to facilitate mate recognition (sensu Paterson 1985); 3) differ among individuals and be repeatable within individuals, to aid individual recognition and mate assessment; and 4) correlate with quality, to allow for mate assessment.

Understanding the function of chemical signals in songbirds is in its very earliest stage of development. Our data demonstrate variability that may be attributed to genetic and physiological differences among individuals and serve as the raw material for evolution. The chemosignals described here also meet several criteria for both mate recognition and mate assessment signals. A mate recognition signal should allow the receiver to identify the appropriate species, sex, and reproductive condition of a potential mate (Johansson and Jones 2007). Volatile compounds have been measured in several songbird species, and the particular compounds identified have differed among all species measured to date (Haribal et al. 2005, 2009; Soini et al. 2007; Soini HA and Whittaker DJ et al., unpublished data). These signals differ significantly between male and female juncos, and the previously described increase in volatile concentration in birds in breeding condition suggests that they communicate information about reproductive condition (Soini et al. 2007). Chemosignals used in mate assessment should have differences between individuals so that each sender has a unique signature (Johansson and Jones 2007), as demonstrated in this study. It has also been suggested that mate assessment signals should be costly to produce, to ensure honesty in the signal, and that they should have a high heritability (Johansson and Jones 2007). The cost of producing volatile compounds in preen oil is currently unknown and could be direct (high metabolic cost) or indirect (attraction of predators or competitors). We have not yet measured heritability of this signal, but the high level of repeatability demonstrated here suggests that heritability may be high (Boake 1989; Johansson and Jones 2007). Alternatively, these chemosignals may not necessarily be important in reproductive behavior and instead may play a role in more general social interactions, for example, within sexes or between parents and offspring (Jones et al. 2004; Hagelin 2007; Johansson and Jones 2007; Whittaker et al. 2009). Additional work is required to test whether juncos are able to discriminate among these odors and whether preferences can be detected; however, in a previous study, we found that juncos can discriminate between preen oil odors from heterospecifics and conspecifics and that they can discriminate between their own preen oil and that of a conspecific (Whittaker et al. 2009).

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

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