

Original Article

Intraspecific preen oil odor preferences in dark-eyed juncos (*Junco hyemalis*)

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Most birds possess a uropygial gland that produces a secretion, preen oil, that contains volatile compounds that may transmit information about individual attributes. However, the ability of passerine songbirds to discriminate among the odors of different individuals has not yet been demonstrated. We tested whether dark-eyed juncos (*Junco hyemalis*) could discriminate among preen oil odors from 1) conspecifics and heterospecifics; 2) male and female conspecifics; 3) conspecifics from same or different population or subspecies; and 4) males with large or small plumage ornaments. Our evidence suggests that juncos can discriminate between the odor of conspecifics and heterospecifics. In 2-way choice tests between conspecific odors, both males and females spent more time with male preen oil. Subjects did not discriminate between odors of individuals from their own population or a different one, and no relationship was found between choice and plumage ornaments. Unexpectedly, females spent more time with the odor of males with smaller body size, regardless of population of origin. This result was especially surprising because an analysis of preen oil volatile compounds revealed that the odors of smaller males were less “male-like” than the odors of larger males. We conclude that songbirds are able to detect odors from preen oil as shown by their ability to distinguish odors arising from males and females. They may also be able to distinguish among individuals of varying body size. *Key words:* birds, chemical communication, mate choice, odor preference, olfaction, passerines, preen oil, Y-maze. [*Behav Ecol* 22:1256–1263 (2011)]

INTRODUCTION

Chemical communication occurs in all organisms, but until relatively recently was mostly ignored in bird species (Wyatt 2003). Chemical cues are used for many purposes, including social behaviors, such as finding, attracting, and evaluating mates (Johansson and Jones 2007). Such behavior is relatively well understood in mammals and insects, but we are only beginning to investigate this mode of communication in birds (Balthazart and Taziaux 2009). Although several studies have demonstrated that olfactory chemical cues play a role in avian ecology and behavior (e.g., Bonadonna and Nevitt 2004; Hagelin 2007; Hirao et al. 2009), few studies have examined the mechanisms associated with cue transmission (but see Zhang et al. 2010). In this study, we examined whether volatile compounds found in avian preen oil (a glandular secretion) could serve as intraspecific communication signals in passerine birds (songbirds), a taxon which has been the subject of very little research on the mechanisms associated with olfaction.

Some birds, particularly seabirds, are known to distinguish odors and to use olfactory cues to find food and nesting sites (reviewed in Balthazart and Taziaux 2009). Evidence for

intraspecific chemical communication has also been found in seabirds (Hagelin et al. 2003) and in a parrot (Zhang et al. 2010). Passerine songbirds were long thought to be the group of birds least likely to have strong olfactory capabilities because they possess the smallest olfactory bulbs relative to brain size among birds as a whole (Bang and Cobb 1968). Recent genomic-level studies, however, have revealed that songbirds have repertoires of olfactory receptors similar to those of seabirds (Steiger et al. 2008, 2009). Further, behavioral studies have shown that passerines are capable of using odor as a navigational cue (Wallraff 2003, 2004), to detect predator odor (Amo et al. 2008; Roth et al. 2008), to find and replace antiparasitic herbs in the nest (Petit et al. 2002), to distinguish between the scent of conspecifics and heterospecifics (Whittaker et al. 2009), and to recognize their natal nests (Caspers and Krause 2010). However, to date, no studies in passerines have examined intraspecific chemical communication, despite the importance of this taxonomic group in studies of social behavior, sexual selection, and speciation.

The primary exocrine gland in most birds is the uropygial gland, or preen gland, which is located above the base of the tail and secretes a substance called preen oil. Birds use their bills to rub this oil over their feathers during preening, where it functions to protect the feathers from environmental degradation and possibly from damage by parasites (Jacob and Ziswiler 1982; Moyer et al. 2003; Douglas 2008). Preen oil also contains volatile compounds that produce an odor (Haribal et al. 2005, 2009; Soini et al. 2007; Whittaker et al. 2010). In some species,

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the odor from this secretion may serve to repel predators (Burger et al. 2004; Hagelin and Jones 2007) or parasites (Douglas 2008). The odor may also be important in communication: Studies have found that the concentrations of preen oil volatile compounds vary seasonally, perhaps in relation to hormonal status (Soini et al. 2007; Douglas et al. 2008; Whelan et al. 2010), and the compounds differ qualitatively among species (Haribal et al. 2005, 2009; Mardon et al. 2010). Bird odor is also variable among individuals and between the sexes, and measurements of individual odor are repeatable within short and long time spans (Bonadonna et al. 2007; Mardon et al. 2010; Whittaker et al. 2010).

In the dark-eyed junco (*Junco hyemalis*), individuals do not differ in the specific compounds present in their preen oil volatile blend, but individuals do differ quantitatively in the relative concentrations of those volatile compounds (Soini et al. 2007; Whittaker et al. 2010). For example, males have higher proportions of the methyl ketones 2-undecanone through 2-pentadecanone, whereas females have higher proportions of the linear alcohol 1-undecanol (Whittaker et al. 2010). Not only do volatile compounds in junco preen oil differ among individuals and between sexes, they also differ between geographically and ecologically distinct populations (Whittaker et al. 2010). Juncos have been shown to discriminate between the odor of conspecifics and heterospecifics as well as between self and other conspecifics in an experimental study of female behavioral response to odors at the nest (Whittaker et al. 2009), but their ability to discriminate quantitative differences between sexes and populations has not been previously tested.

Studies in both birds and mammals indicate that animals choose mates or evaluate rivals nonrandomly, and in mammals, volatile compounds in urine or glandular secretions are known to function as reliable signals of individual traits (e.g., Singer et al. 1997; Novotny et al. 2007; Charpentier et al. 2008, 2010; Kwak et al. 2009). Because of the information contained in volatile compounds in avian preen oil, these odors are potentially useful for individual recognition and assessment (Johansson and Jones 2007). If, for example, the odors are important for mate attraction, we would predict that birds will show preferences for odors from the same species, opposite sex, and, to the extent that they mate preferentially by population of origin, they should show preference for individuals of their own population. Further, if these odors are important in mate assessment, we predict that birds would prefer the odor of individuals that also possess visual indicators of quality, such as plumage ornaments. In songbirds, the size or intensity of color of plumage ornaments may be related to overall body size (McGlothlin et al. 2005), steroid hormone levels (Gonzalez et al. 2001; McGlothlin et al. 2008), immune function (Nolan et al. 2006), and other aspects of quality (Hill 1991). Like plumage ornaments, individual differences in odor could also be related to steroid hormones (Douglas et al. 2008; Whittaker DJ, Soini HA, Gerlach NM, Posto AL, Novotny MV, and Ketterson ED, unpublished data), immune function, or major histocompatibility complex genotype (Singer et al. 1997; Setchell et al. 2011). Thus, we hypothesize that plumage ornaments and odor may convey information about an individual's quality.

We tested the ability of dark-eyed juncos to differentiate between preen oil odors from different species, different sexes, and from subspecies or populations that have geographically separate breeding ranges by presenting captive juncos with a 2-way choice test. We predicted that juncos in breeding condition would prefer the odor of birds of the opposite sex and that they would prefer the odor of birds from their own subspecies or population. We also tested the ability of female juncos to differentiate between the odor of males with large or

small amounts of white in the outer rectrices ("tail-white," a plumage ornament that is attractive to females, Hill et al. 1999), with the prediction that they would prefer the odor of males with greater amounts of tail-white.

METHODS

Study subjects

Our subjects were dark-eyed juncos (*Junco hyemalis*, Family: Emberizidae), a widespread North American sparrow, held at Kent Farm Bird Observatory at Indiana University in Bloomington, Indiana. The morphology, behavior, and physiology of this species are well understood, and the bird has previously been the subject of avian chemical communication studies (Soini et al. 2007; Whittaker et al. 2009, 2010). The junco rapidly diverged over the last 10 000–100 000 years into several geographically and morphologically distinct subspecies (Milá et al. 2007), 3 of which were included in the present study: the Carolina junco, the white-winged junco, and the Oregon junco.

The Carolina junco (*J. h. carolinensis*) subjects were from the Mountain Lake population in Virginia, a site that has been the location of long-term research on junco behavior and physiology for over 30 years (Ketterson et al. 2001), and were brought into captivity as adults in the fall of 2009. The white-winged junco (*J. h. aikeni*) is found in the Black Hills in South Dakota, and the subjects used in this study were brought into captivity in May 2010. Two very recently diverged populations of the Oregon junco (*J. h. thurberi*) from San Diego County, California were also the subject of study. Dark-eyed juncos colonized the urban campus of the University of California at San Diego (UCSD), a novel breeding environment for this species, in the early 1980s, becoming sedentary (Yeh 2004) and diverging from the populations in the nearby ancestral-range population in the Laguna Mountains in morphology, genetics, and behavior (Rasner et al. 2004; Yeh 2004; Yeh and Price 2004; Newman et al. 2006; Yeh et al. 2007; Price et al. 2008). Juncos from the UCSD and Laguna Mountain populations were captured as independent juveniles in the summer of 2007 and brought into captivity as part of a common-garden study (Atwell JW, unpublished data). These 2 populations display significant differences in preen oil volatile compound blends (Whittaker et al. 2010). The 3 subspecies in the study differ in body size and plumage (summarized in Table 1). Junco subspecies are likely capable of interbreeding and do hybridize in contact zones, but they are in the process of diverging and perhaps moving toward reproductive isolation (Milá et al. 2007).

All juncos were housed in an indoor aviary, with light cycles mimicking the natural light/dark cycle for the date. Birds from

Table 1
Average measurements of juncos used in this study

Population	Mass	Wing	Tail	Tarsus	Tail-white
Females					
Virginia	22.40	77.50	68.10	22.20	1.90
South Dakota	22.80	85.10	70.40	22.00	3.20
Laguna Mountain	18.11	75.67	67.28	20.00	2.28
UCSD	18.13	73.63	66.10	19.87	1.89
Males					
Virginia	23.00	82.60	73.30	22.50	2.30
South Dakota	23.70	90.70	76.30	21.50	3.21
Laguna Mountain	19.15	79.08	71.46	20.25	2.56
UCSD	16.83	77.92	69.71	20.09	2.16

Mass is in grams; wing, tail, and tarsus length are in millimeters. Tail-white score is the sum of the proportion of white on each rectrix.

Virginia and California were housed in free-flying aviary rooms with birds from their own populations, whereas birds from South Dakota were housed in individual cages. Birds were fed ad libitum on millet, sunflower seeds, orange slices, and a mixture of puppy chow, hardboiled eggs, and carrots. All subjects were in breeding condition, as determined by observations of brood patches in females and cloacal protuberances in males, at the time of the trials, which occurred in June–July 2009 and June–July 2010.

Morphological measurements

We weighed each subject and preen oil donor (birds that contributed preen oil samples for the behavioral assays) to the nearest 0.1 g with a Pesola spring balance, measured wing length to the nearest 1 mm, and tarsus length to the nearest 0.1 mm (Table 1). We measured tail-white visually, by estimating the proportion of each outer tail-feather that is white. Visual estimation has been found to be highly correlated (e.g., >95%) with computer image analysis and is considered very reliable (McGlothlin et al. 2005).

Preen oil collection

All preen oil samples were collected by gently rubbing the preen gland with a capillary tube; this action stimulates the gland to secrete up to 5 mg of preen oil which is then collected in the tube (Whittaker et al. 2009, 2010). Junco preen oil samples were collected from captive birds at Kent Farm Bird Observatory and from wild birds at Mountain Lake Biological Station in Virginia. For heterospecific preen oil samples, we collected preen oil from brown-headed cowbirds (*Molothrus ater*), a brood parasite that regularly parasitizes junco nests at our field sites. All samples were weighed and stored at -20°C until used in the experiment.

Preen oil presentation

To present preen oil odors, we dissolved preen oil in pure acetone (1 mg preen oil per 100 μl acetone). We then applied 50 μl of the solution to either a clean empty food dish (habituation/discrimination experiments) or a cotton ball (Y-maze trials). This method was used successfully in a previous field experiment (Whittaker et al. 2009). Birds were exposed to odors from unfamiliar individuals with one exception—the California birds that were sampled from the same population (UCSD or Laguna) were familiar to one another.

Habituation/discrimination trials

In 2009, we ran preliminary odor discrimination trials using the habituation/discrimination paradigm (Halpin 1974). In this paradigm, the subject is first presented with one odor (Odor A) and given time to habituate to the presence of this odor (habituation phase). The subject is then presented with 2 odors simultaneously: Odor A, and Odor B that differs from Odor A (discrimination phase). If the subject spends more time investigating odor B than odor A during the second phase of the trial, the subject is considered able to discriminate between these 2 odors. Using this paradigm, we tested whether juncos could discriminate between preen oil odors from conspecifics and heterospecifics and from same- and opposite-sex conspecifics.

For these trials, we used UCSD and Laguna Mountain juncos as the subjects, individually housed in cages. The subjects' food dishes were removed from their home cage 30 min before the start of the trial. Odors were then presented in

clean empty food dishes in the corners of the cage. In addition to the presented odor(s), we also presented dishes that had 50 μl of pure acetone applied and 1 or 2 blank dishes as controls, so that all 4 corners of the cage had a dish. We randomized the location of the presented odors in each phase of each trial. The habituation phase lasted 20 min, whereas the discrimination phase lasted for 10 min. Due to time constraints, we only tested 1 hypothesis on each sex. A previous study indicated that female juncos could distinguish between the odor of conspecifics and heterospecifics in their nest but used preen oil from mockingbirds for the heterospecific odor—a scent that juncos would not typically encounter during the breeding season (Whittaker et al. 2009). In this study, we tested female subjects' ability to discriminate between conspecifics (Odor A) and heterospecifics (Odor B), using preen oil from a more ecologically relevant species—female brown-headed cowbirds, a species that regularly parasitizes junco nests. As a preliminary test of whether juncos could discriminate within species between the sexes, we presented male subjects with preen oil from a male conspecific (Odor A) and a female conspecific (odor B).

Y-maze trials

In 2010, we employed a Y-maze odor preference test following Hagelin et al. (2003) and Zhang et al. (2010), with minor modifications. We used a Y-maze constructed of clear plexiglass (height 20 cm [8"], arms 40 cm [16"] long by 20 cm [8"] wide, and a neutral central area 35 cm [14"] long by 20 cm [8"] wide) (Figure 1). To avoid repeated attempts by subjects to escape through the plexiglass top, we modified it by attaching a mesh cover so that the subjects could detect the ceiling. Because passerines are usually uncomfortable standing on flat surfaces, we placed perches at the end of each arm of the maze, and between the perches and the end of the arm, we placed the odor stimuli. We controlled airflow by using a vacuum pump (Neptune Dyna-Pump, model AB1PO23N; Fisher Scientific, Pittsburgh, PA) to pull air from the arms out of the maze; the openings for the pump were placed behind the perches, toward the start of the maze, so that odor from the stimulus would be pulled toward the perch, but the 2 odors would not mix in the middle of the maze. The air pull could be felt near the openings, but it was weak enough that we could not detect a physical sensation of airflow further back in the maze, and it did not appear to disturb the birds.

About 2 weeks prior to the start of the trials, each subject was given 30 min to habituate to the maze, with food dishes placed at the end of each arm of the Y to encourage exploration. Birds were housed in individual cages beginning the night before the start of the trials. The following morning,

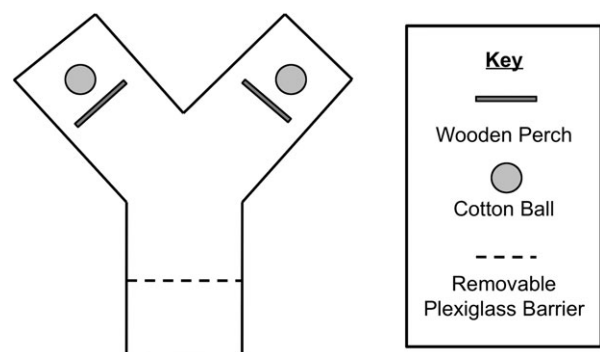


Figure 1
Diagram of plexiglass Y-maze used in odor preference trials.

the birds were captured by hand in their cages in the dark to reduce stress and placed in the start box in the Y-maze (which was housed in a separate room). The subject was given 5 min in the start box to habituate to being enclosed in the maze, and then the experimenter lifted the barrier and the subject was allowed to explore the maze for 10 min. At the end of the trial, the lights were turned off, and the bird was captured by hand and returned to its home cage. The maze was wiped thoroughly with 70% ethanol between each trial and allowed to air dry.

We ran 3 sets of trials, based on our hypotheses:

- 1) Sex preference: subjects were given a choice between the odor of a same-sex, same-population individual or the odor of an opposite-sex, same-population individual.
- 2) Population preference: subjects were given a choice between the odor of an opposite-sex individual from their own population or the odor of an opposite-sex individual from a different population. The 4 populations were paired: Carolina juncos chose between preen oil from Carolina juncos and white-winged juncos and vice versa; UCSD and Laguna Mountain juncos were paired in the same way.
- 3) Tail-white preference: female subjects were given a choice between the odor of a male with a high amount of tail-white and the odor of a male with a low amount of tail-white; both males were from the same population as the subject.

The sex preference trials did not include the white-winged juncos as subjects because they were brought into captivity later in the study. All subjects in the sex preference trials plus the white-winged juncos were used in the population preference trials; all females from the population preference trials were used in the tail-white preference trials. A minimum of 2 weeks was allowed between each trial for a given individual.

Video analysis

All trials were videotaped. The habituation/discrimination videos were scored by one experimenter (A.K.M.), who was blind to the subject identity and treatment while scoring, using the software ODLog (Macropod Software). Because these birds were very active during the day, we chose to use an instantaneous sampling technique and record their location (in each quadrant of the cage as well as on perches, which were considered a neutral area) every 10 s (Martin and Bateson 1993). We analyzed the percent of these scan samples spent in each quadrant of the cage. The Y-maze preference trials were scored using ODLog by 2 experimenters (K.M.R. and assistant Elizabeth Swanger), who were blind to identity and treatment of each bird, for time spent in the neutral area (middle and start box) and in each arm of the maze. We were able to use a continuous sampling technique in the Y-maze trials, and we analyzed the total amount of time spent in each arm of the maze.

Statistical analysis

We tested all data for normality using the Shapiro–Wilk test in SPSS. All datasets differed significantly from a normal distribution, so we used the nonparametric Wilcoxon signed-ranks test to compare the amount of time spent near each presented preen oil odor. All statistical tests were 2 tailed.

A total of 22 trials were run using the habituation/discrimination paradigm (9 using junco/cowbird preen oil, 13 using male/female junco preen oil). A total of 201 Y-maze trials were run (71 sex preference, 91 population preference, and 39 within-population tail-white preference). In the analysis, we only included trials in which the subjects entered both arms of the maze or spent at least 10 s in the neutral area in addition to one arm of the maze, in order to meet the assumption that the subjects were able to detect both odors. We also

removed trials in which the cotton balls were disturbed enough that they were moved out of the arm of the Y-maze, typically as a result of the subject flapping its wings. We analyzed a total of 112 Y-maze trials. We detected no bias in the deleted trials: In the trials in which the subject spent the entire trial in only one arm of the maze, 54% went to the right arm, 46% went to the left arm; 52% went to the arm containing the odor of the same sex or same population, whereas 57% went to the opposite sex or different population.

RESULTS

Habituation/discrimination trials

During the discrimination phase of the trials, the subjects spent $8.81 \pm 1.76\%$ (mean \pm standard error mean) of the trial with Odor A (the habituation odor) and $16.84 \pm 3.86\%$ with Odor B (Figure 2). The results suggest a trend toward more time spent with the unfamiliar odor, but the results are not statistically significant (Wilcoxon signed-rank test, $P = 0.073$, $n = 22$). If we analyze the 2 sexes separately, the results suggest females may discriminate between conspecifics and heterospecifics ($P = 0.093$, $n = 9$), whereas males do not appear to discriminate between male and female preen oil odor ($P = 0.345$, $n = 13$).

Y-maze trials: sex preference

We analyzed 40 trials (22 male subjects, 18 female subjects). The hypothesis that subjects would show a preference for preen oil odor from the opposite sex was not supported. Instead, subjects of both sexes displayed a significant, nonrandom preference for male preen oil, spending 247.5 ± 32.5 s with male preen oil and 127.48 ± 22.0 s with female preen oil (Wilcoxon signed-rank test, $P = 0.024$, Figure 3). The trend was even stronger for male subjects, who spent 274.3 ± 42.8 s with male preen oil and only 93.1 ± 17.2 s with female preen oil ($P = 0.003$).

Y-maze trials: population preference

We analyzed 51 trials (30 male subjects, 21 female subjects). We hypothesized that all subjects would prefer the odor of the preen oil from their own population. The results do not support this hypothesis. Males showed no preference (Figure 4A), whereas female preference varied depending on the subject's own population (Figure 4B). When we looked closely at which

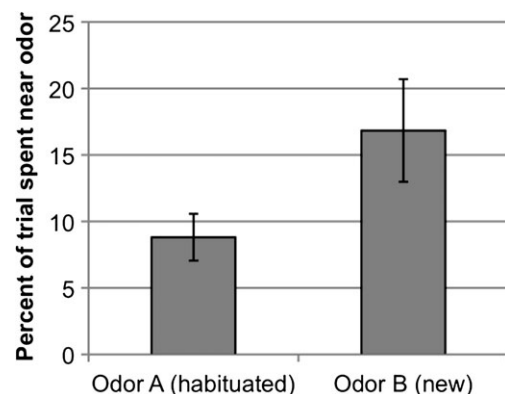


Figure 2

Results of habituation/discrimination trials, in which we tested the ability of subjects to discriminate between either 1) a heterospecific (brown-headed cowbird) and a conspecific or 2) a male and a female conspecific.

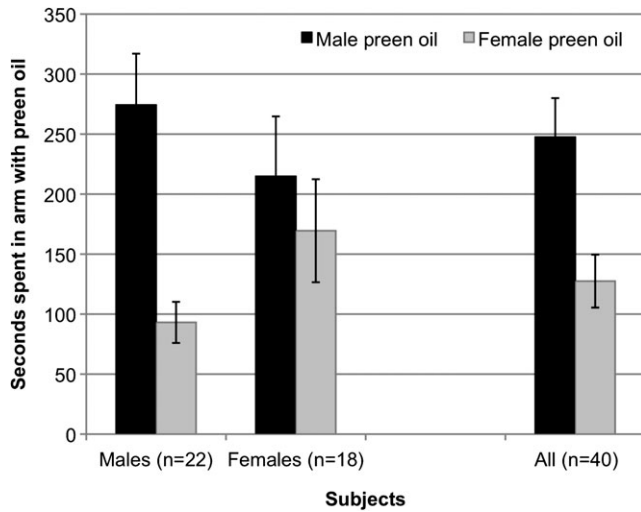


Figure 3
Results of Y-maze trials in which subjects chose between male or female preen oil.

populations the female subjects preferred, however, we noticed an unexpected pattern: Females were choosing the preen oil from the population that was characterized by smaller body size and lower amounts of tail-white (Figure 5). Specifically, Carolina junco and white-winged junco females

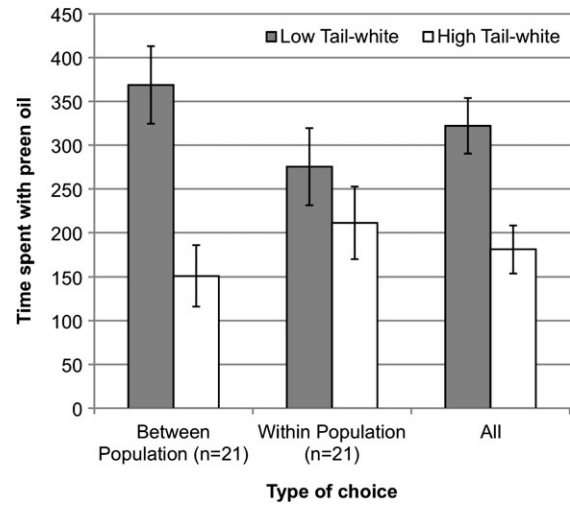


Figure 5
Results of Y-maze trials; females choosing between males with low or high tail-white (within and between populations).

preferred preen oil odors from Carolina junco males (Carolina junco preen oil, 384.6 ± 78.4 s vs. white-winged junco preen oil, 129.9 ± 64.0 s), and females from the UCSD and Laguna Mountain populations preferred the preen oil odors from UCSD males (UCSD preen oil, 356.8 ± 53.5 s vs. Laguna Mountain preen oil, 166.7 ± 39.6 s). The preference for odors from these 2 populations, in which juncos have smaller body size and lower amounts of tail-white, was significant (Wilcoxon signed-rank test, $P = 0.012$).

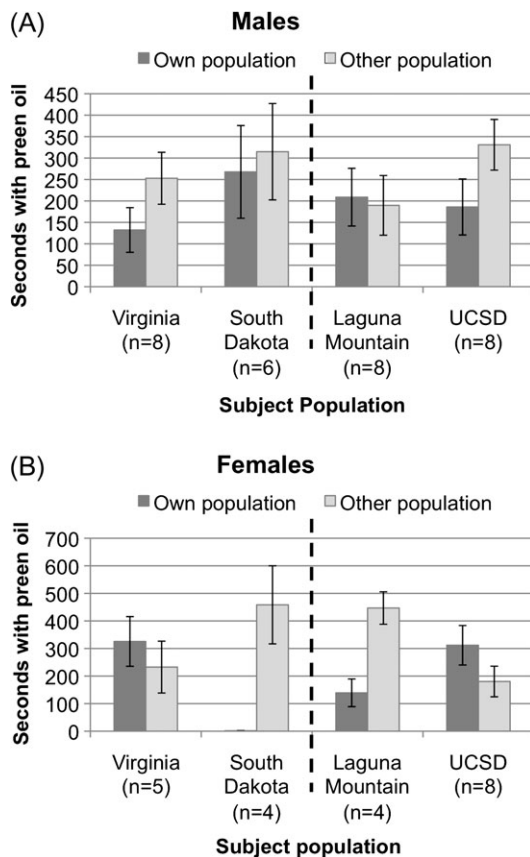


Figure 4
Results of Y-maze trials: choice of opposite sex from same population or from different population. Dashed line divides the sets of populations that were paired together. (A) Choices made by males for scent of females; (B) choices made by females for scent of males.

Y-maze trials: within-population tail-white preference

When we gave females the choice between high tail-white and low tail-white males from their own population, they showed no preference (275.4 ± 44.1 s, low tail-white, vs. 211.2 ± 27.2 s, high tail-white, $n = 21$, $P = 0.414$, Figure 5).

Post hoc analysis: preferences for body size, body condition, and tail-white

We conducted post hoc analyses of both the between-population choice trials and the intrapopulation tail-white choice trials (described above) comparing the tail-white scores (a static measure of individual quality, Hill et al. 1999), body size (as measured by wing length, McGlothlin et al. 2005), and body condition (ratio of mass to tarsus length, a dynamic measure of quality, Hill et al. 1999) of the donors of the “preferred” and “unpreferred” male preen oil odors (Figure 6). We found that the males that donated the preferred preen oil odors were significantly “smaller” than the males that donated the preen oil odors that subjects avoided (Wilcoxon signed-ranks test, $P = 0.013$ across all trials; within population, $P = 0.087$; between populations, $P = 0.067$). The same relationship was observed for mass/tarsus ratio, with females preferring the odor of males with a lower ratio, though this trend was significant only in the between-population trials ($P = 0.08$ across all trials; within population, $P = 0.945$; between populations, $P = 0.044$). Differences in tail-white score of preferred versus unpreferred males were not significant ($P = 0.197$ across all trials; within population, $P = 0.639$; between populations, $P = 0.234$).

The relationship between body size and preen oil volatile compounds has not been previously analyzed. To investigate whether a relationship was present, we reanalyzed the dataset

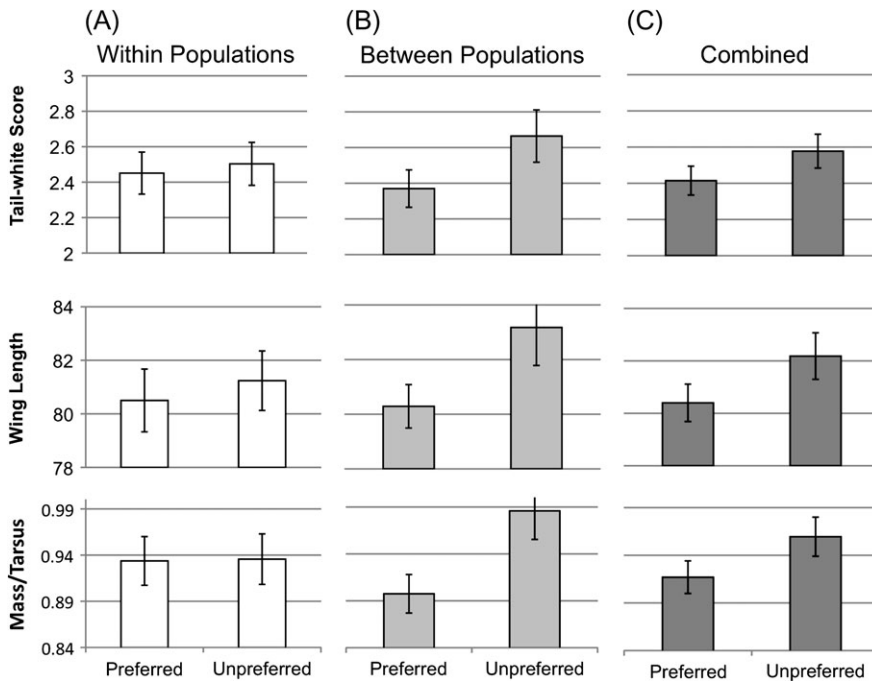


Figure 6 Summary of tail-white, body size (wing length), and body condition (mass/tarsus ratio) measurements of males that contributed preferred and unpreferred odors in within-population trials (A), between-population trials (B), and within- and between-population results combined (C). Male wing length was the most significant predictor of female odor preference.

from Whittaker et al. (2010), which tested for a relationship between volatile compounds and identity, sex, and population of origin in dark-eyed juncos from California. We found a strong positive correlation between wing length and principal component 1 (PC1) (Pearson correlation = 0.692, $P < 0.001$). Details of the principal components analysis can be found in Whittaker et al. (2010). In the original results, PC1 was negatively correlated with proportions of linear alcohols 1-decanol and 1-undecanol and positively correlated with proportions of the 5 methyl ketones 2-undecanone through 2-pentadecanone; males have significantly higher PC1 scores than females (Whittaker et al. 2010). In the present reanalysis, larger males have higher PC1 scores and thus have a more “male-like” odor.

DISCUSSION

These data suggest that songbirds are able to discriminate between different preen oil odors and that they can differentiate between male and female odor and between odors from birds with varying body size. However, the preferences exhibited by the subjects in this study were not in the direction that we predicted. We predicted that juncos in breeding condition would prefer the odor of the opposite sex; instead, both males and females preferred the odor of males. We also predicted that juncos in breeding condition would prefer the odor of opposite-sex individuals from their own population over those from a different population. Males showed no preference for females from their own or other population. Females, however, showed preferences for males from particular populations but not necessarily their own; the preferred preen oil odors were donated by males from populations characterized by smaller body size and lower amounts of tail-white. Finally, we predicted that females would prefer the odor of males with higher tail-white scores or larger body sizes. We found no significant support for the association of odors with degree of ornamentation. However, we found that females spent significantly more time near the preen oil of males with smaller body size. To test whether a relationship exists between body size and odor, we reanalyzed data from Whittaker et al. (2010) and found a significant correlation between preen oil volatile

compounds and body size—larger males have more male-like odor.

Although we expected that subjects would prefer the odor of the opposite sex, we found that both sexes were attracted to male odor. Given the mating system of juncos—territorial monogamous pairs, with moderate frequency of extrapair fertilizations (~25% of all offspring, Ketterson et al. 1997), it is reasonable to suggest that whereas females may be primarily interested in investigating potential mates, males may be more motivated to fend off potential rivals. This preference for male odor may also explain the males’ failure to spend more time with the discrimination odor (female preen oil) in the habituation/discrimination trials.

Interpreting odor preferences in a 2-way choice test is not straightforward—odor preferences may not indicate mate preference. The observed preference for male odors by both sexes may be associated with affiliative behavior, aggression, or territorial responses, or dominance interactions, rather than a mate assessment or preference response. Also, apparent preference for one odor may actually be avoidance of the other odor; instead of preferring the odor of smaller or less ornamented males, females may be interpreted to be actively avoiding the odor of larger, more ornamented males, which could be perceived as more aggressive, threatening, or dominant, as discussed below.

If odor preference is indicative of mate preference, these data suggest that juncos from morphologically distinct populations or subspecies may not use chemosignals to recognize other populations as a different group, but instead only evaluate other individuals along a continuum of shared junco traits. In fact, juncos may prefer traits that are not found in the continuum of their own population. For example, in a different study, females preferred males with experimentally increased tail-white, although the tail-white scores of the experimental males were well above the range normally encountered in natural males (Hill et al. 1999). Although all juncos have tail-white, the subspecies vary in average size of the white patch (e.g., Table 1); females may actually prefer tail-white patches more typical of a different population from their own. Similarly, females may prefer odors with a relative volatile compound concentration that is outside

the range found in their own populations. As noted previously, junco populations differ quantitatively in their volatile compound concentrations, but not qualitatively (Whittaker et al. 2010).

Alternatively, the time spent near an odor could be attributed to a territorial or aggressive response to an unfamiliar odor or to an interest in a novel or unfamiliar odor. In another study, Laguna Mountain juncos showed a more aggressive response than UCSD juncos to song playbacks (Newman et al. 2006), and preliminary evidence suggests that white-winged juncos have a stronger territorial response than Carolina juncos in Virginia (Bergeon Burns C, unpublished data). The apparent "preference" of females from Laguna Mountain and South Dakota for male preen oil from a different population may be an aggressive response, in the same way that we might interpret male preference for male odor to be aggressive. On the other hand, a previous study with UCSD and Laguna Mountain juncos found that UCSD juncos exhibited significantly faster and more extensive early exploratory behavior than the Laguna Mountain juncos (Atwell JW, unpublished data). In the present study, the pattern observed is the opposite: UCSD females spent more time near familiar same-population preen oil odors, whereas the Laguna Mountain females spent more time near the unfamiliar different-population preen oil odors. Additionally, the birds from Virginia and South Dakota were all given the choice of preen oil samples from 2 unfamiliar individuals. Thus, we suggest that it is unlikely that pattern of female preferences can be explained by a preference for unfamiliar odors and that more likely the birds are expressing a preference for the odor of males with smaller body size (or avoidance of larger males) or other related phenotypic traits.

The rapid phenotypic diversification of dark-eyed junco populations (Milá et al. 2007) and their allopatric distribution, combined with limited hybridization along contact zones (Nolan et al. 2002), has been interpreted to suggest that *Junco hyemalis* may be a case of incipient speciation (Milá et al. 2007). However, the data presented here suggest that, at least in the case of chemical cues, dark-eyed junco individuals may not recognize differences between subspecies or populations and instead evaluate the traits of a potential mate along a species-wide continuum.

In conclusion, this study provides the first direct experimental test of whether songbirds can detect preen oil odors and use them to discriminate among individual conspecifics that differ in sex, population of origin, or morphological traits. Our evidence suggests that songbirds can use preen oil to choose non-randomly among sexes and individuals, but the behavioral context (e.g., mate choice vs. social avoidance) motivating these choices was not clear from our data. This study builds on a growing body of evidence suggesting that odor is an important signaling mode in the behavioral ecology of songbirds, and particularly that preen oil is an important candidate for continued experimental and observational work in songbirds.

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REFERENCES

- Amo L, Galván I, Tomás G, Sanz JJ. 2008. Predator odour recognition and avoidance in a songbird. *Funct Ecol*. 22:289–293.
- Balthazart J, Taziaux M. 2009. The underestimated role of olfaction in avian reproduction? *Behav Brain Res*. 200:248–259.
- Bang BG, Cobb S. 1968. The size of the olfactory bulb in 108 species of birds. *Auk*. 85:55–61.
- Bonadonna F, Miguel E, Grosbois V, Jouventin P, Bessiere J-M. 2007. Individual odor recognition in birds: an endogenous olfactory signature on petrel's feathers? *J Chem Ecol*. 33:1819–1829.
- Bonadonna F, Nevitt GA. 2004. Partner-specific odor recognition in an Antarctic seabird. *Science*. 306:835.
- Burger BV, Reiter B, Borzyk O, du Plessis MA. 2004. Avian exocrine secretions. I. Chemical characterization of the volatile fraction of the uropygial secretion of the green woodhoopoe, *Phoeniculus purpureus*. *J Chem Ecol*. 30:1603–1611.
- Caspers BA, Krause ET. 2010. Odour-based natal nest recognition in the zebra finch (*Taeniopygia guttata*), a colony-breeding songbird. *Biol Lett*. 7:184–186.
- Charpentier MJE, Boulet M, Drea CM. 2008. Smelling right: the scent of male lemurs advertises genetic quality and relatedness. *Mol Ecol*. 17:3225–3233.
- Charpentier MJE, Crawford JC, Boulet M, Drea CM. 2010. Message "scent": lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues. *Anim Behav*. 80:101–108.
- Douglas HD 3rd. 2008. Prenuptial perfume: alloantoining in the social rituals of the crested auklet (*Aethia cristatella*) and the transfer of arthropod deterrents. *Naturwissenschaften*. 95:45–53.
- Douglas HD 3rd, Kitaysky AS, Kitaiskaia EV. 2008. Seasonal variation in progesterone and odorant emissions among breeding crested auklets (*Aethia cristatella*). *Horm Behav*. 54:325–329.
- Gonzalez G, Sorci G, Smith LC, de Lope F. 2001. Testosterone and sexual signalling in male house sparrows (*Passer domesticus*). *Behav Ecol Sociobiol*. 50:557–562.
- Hagelin JC. 2007. The citrus-like scent of crested auklets: reviewing the evidence for an avian olfactory ornament. *J Ornithol*. 148: S195–S201.
- Hagelin JC, Jones IL. 2007. Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication? *Auk*. 124:741–761.
- Hagelin JC, Jones IL, Rasmussen LEL. 2003. A tangerine-scented social odour in a monogamous seabird. *Proc R Soc Ser B Biol Sci*. 270:1323–1329.
- Halpin ZT. 1974. Individual differences in the biological odors of the Mongolian gerbil (*Meriones unguiculatus*). *Behav Biol*. 11:253–259.
- Haribal M, Dhondt AA, Rodriguez E. 2009. Diversity in chemical compositions of preen gland secretions of tropical birds. *Biochem Syst Ecol*. 37:80–90.
- Haribal M, Dhondt AA, Rosane D, Rodriguez E. 2005. Chemistry of preen gland secretions of passerines: different pathways to the same goal? why? *Chemoecology*. 15:251–260.
- Hill GE. 1991. Plumage coloration is a sexually selected indicator of male quality. *Nature*. 350:337–339.
- Hill JA, Enstrom DE, Ketterson ED, Nolan V Jr, Ziegenfus C. 1999. Mate choice based on static vs. dynamic secondary sexual traits in the dark-eyed junco. *Behav Ecol*. 10:91–96.
- Hirao A, Aoyama M, Sugita S. 2009. The role of uropygial gland on sexual behavior in domestic chicken *Gallus gallus domesticus*. *Behav Processes*. 80:115–120.
- Jacob JP, Ziswiler V. 1982. The uropygial gland. In: Farner DS, King JR, Parkes KC, editors. *Avian biology*. New York: Academic Press. p. 199–324.
- Johansson BG, Jones TM. 2007. The role of chemical communication in mate choice. *Biol Rev*. 82:265–289.

- Ketterson ED, Nolan V Jr, Casto JM, Buerkle CA, Clotfelter ED, Grindstaff JL, Jones KJ, Lipar JL, McNabb FMA, Neudorf DLH, et al. 2001. Testosterone, phenotype, and fitness: a research program in evolutionary behavioral endocrinology. In: Dawson A, Chaturvedi CM, editors. Avian endocrinology. New Delhi (India): Narosa Publishing House. p. 19–40.
- Ketterson ED, Parker PG, Raouf SA, Nolan V Jr, Ziegenfus C, Chandler CR. 1997. The relative impact of extra-pair fertilizations on variation in male and female reproductive success in dark-eyed juncos (*Junco hyemalis*). Ornithol Monogr. 1997:81–101.
- Kwak J, Opiekun MC, Matsumura K, Preti G, Yamazaki K, Beauchamp GK. 2009. Major histocompatibility complex-regulated odortypes: peptide-free urinary volatile signals. Physiol Behav. 96:184–188.
- Mardon J, Saunders SM, Anderson MJ, Couchoux C, Bonadonna F. 2010. Species, gender, and identity: cracking petrels' sociochemical code. Chem Senses. 35:309–321.
- Martin P, Bateson P. 1993. Measuring behavior: an introductory guide. 2nd ed. Cambridge (UK): Cambridge University Press.
- McGlothlin JW, Jawor J, Greives TJ, Casto JM, Phillips JL, Ketterson ED. 2008. Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. J Evol Biol. 21:39–48.
- McGlothlin JW, Parker PG, Nolan V Jr, Ketterson ED. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. Evolution. 59:658–671.
- Milá B, McCormack JE, Castaneda G, Wayne RK, Smith TB. 2007. Recent postglacial range expansion drives the rapid diversification of a songbird lineage in the genus *Junco*. Proc R Soc Lond B Biol Sci. 274:2653–2660.
- Moyer BR, Rock AN, Clayton DH. 2003. Experimental test of the importance of preen oil in rock doves (*Columba livia*). Auk. 120:490–496.
- Newman MM, Yeh PJ, Price TD. 2006. Reduced territorial responses in dark-eyed juncos following population establishment in a climatically mild environment. Anim Behav. 71:893–899.
- Nolan PM, Dobson FS, Dresch B, Jouventin P. 2006. Immunocompetence is signalled by ornamental colour in king penguins, *Aptenodytes patagonicus*. Evol Ecol Res. 8:1325–1332.
- Nolan V Jr, Ketterson ED, Cristol DA, Rogers CM, Clotfelter ED, Titus R, Schoech SJ, Snajdr E. 2002. Dark-eyed junco (*Junco hyemalis*). Philadelphia (PA): The Birds of North America, Inc.
- Novotny MV, Soini HA, Koyama S, Wiesler D, Bruce KE, Penn D. 2007. Chemical identification of MHC-influenced volatile compounds in mouse urine. I: Quantitative proportions of major chemosignals. J Chem Ecol. 33:417–434.
- Petit C, Hossaert-McKey M, Perret P, Blondel J, Lambrechts MM. 2002. Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecol Lett. 5:585–589.
- Price TD, Yeh PJ, Harr B. 2008. Phenotypic plasticity and the evolution of a socially selected trait following colonization of a novel environment. Am Nat. 172:S49–S62.
- Rasner CA, Yeh P, Eggert LS, Hunt KE, Woodruff DS, Price TD. 2004. Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. Mol Ecol. 13:671–681.
- Roth TC 2nd, Cox JG, Lima SL. 2008. Can foraging birds assess predation risk by scent? Anim Behav. 76:2021–2027.
- Setchell JM, Vaglio S, Abbott KM, Moggi-Cecchi J, Boscaro F, Pieraccini G, Knapp LA. 2011. Odour signals major histocompatibility complex genotype in an Old World monkey. Proc R Soc Lond B Biol Sci. 278:274–280.
- Singer AG, Beauchamp GK, Yamazaki K. 1997. Volatile signals of the major histocompatibility complex in male mouse urine. Proc Natl Acad Sci U S A. 94:2210–2214.
- Soini HA, Schrock SE, Bruce KE, Wiesler D, Ketterson ED, Novotny MV. 2007. Seasonal variation in volatile compound profiles of preen gland secretions of the dark-eyed junco (*Junco hyemalis*). J Chem Ecol. 33:183–198.
- Steiger SS, Fidler AE, Valcu M, Kempenaers B. 2008. Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? Proc R Soc Lond B Biol Sci. 275:2309–2317.
- Steiger SS, Kuryshv VY, Stensmyr MC, Kempenaers B, Mueller JC. 2009. A comparison of reptilian and avian olfactory receptor gene repertoires: species specific expansion of group γ genes in birds. BMC Genomics. 10:446.
- Wallraff HG. 2003. Olfactory navigation by birds. J Ornithol. 144:1–32.
- Wallraff HG. 2004. Avian olfactory navigation: its empirical foundation and conceptual state. Anim Behav. 57:189–204.
- Whelan RJ, Levin TC, Owen JC, Garvin MC. 2010. Short-chain carboxylic acids from gray catbird (*Dumetella carolinensis*) uropygial secretions vary with testosterone levels and photoperiod. Comp Biochem Physiol B Biochem Mol Biol. 156:183–188.
- Whittaker DJ, Reichard DG, Dapper AL, Ketterson ED. 2009. Behavioral responses of nesting female dark-eyed juncos *Junco hyemalis* to hetero- and conspecific passerine preen oils. J Avian Biol. 40:579–583.
- Whittaker DJ, Soini HA, Atwell JW, Hollars C, Novotny MV, Ketterson ED. 2010. Songbird chemosignals: preen oil volatile compounds vary among individuals, sexes, and populations. Behav Ecol. 21:608–614.
- Wyatt TD. 2003. Pheromones and animal behaviour: communication by smell and taste. Cambridge (UK): Cambridge University Press.
- Yeh PJ. 2004. Rapid evolution of a sexually selected trait following population establishment in a novel habitat. Evolution. 58:166–174.
- Yeh PJ, Hauber ME, Price TD. 2007. Alternative nesting behaviours following colonisation of a novel environment by a passerine bird. Oikos. 116:1473–1480.
- Yeh PJ, Price TD. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. Am Nat. 164:531–542.
- Zhang J-X, Wei W, Zhang J-H, Yang W-H. 2010. Uropygial gland-secreted alkanols contribute to olfactory sex signals in budgerigars. Chem Senses. 35:375–382.