

## Behavioral responses of nesting female dark-eyed juncos *Junco hyemalis* to hetero- and conspecific passerine preen oils

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Several studies have suggested a greater role for olfactory cues in avian social interactions than previously recognized, but few have explicitly investigated the effect of odor on parental behavior. We present results from a preliminary study in which we applied hetero- and conspecific preen gland secretions, which are known to contain volatile compounds, to the nests and eggs of incubating female dark-eyed juncos *Junco hyemalis*. The responses to these two conditions were compared to the responses of females whose nests were treated with their own preen oil as a control condition, and to females whose nests were treated with the vehicle only. We found that females significantly reduced incubation bout length, a form of parental care, in response to alien secretions, more so if they came from a heterospecific than a conspecific. Females did not reduce incubation bout length in response to their own preen oil or to a vehicle-only control. These results suggest that odors in the nest may influence avian parental care. However, the behavioral change was only temporary and had no effect on later hatching success. In our study population, brood parasitism by brown-headed cowbirds is common, but resulting nest abandonment is rare; juncos are frequently able to successfully breed even with cowbird nestlings in their nests. Thus, we suggest that more extreme behavioral responses to alien odor, such as nest abandonment or egg ejection, may not be adaptive and should not be expected.

The role of olfaction in bird behavior has been considered in a number of contexts (reviewed in Roper 1999, Hagelin and Jones 2007), including foraging (e.g., Nevitt et al. 1995, 2008), nest building (Petit et al. 2002), navigation (Wallraff 2004), reproductive behavior (Hagelin et al. 2003, Balthazart and Taziaux 2009), and individual recognition (Bonadonna et al. 2003, De Leon et al. 2003, Bonadonna and Nevitt 2004, Bonadonna et al. 2007). Much of this research has focused on seabirds in the orders Charadriiformes and Procellariiformes, but the ability to detect odors has also been demonstrated in passerine species (Clark et al. 1993). Songbirds may respond adaptively to the odor of predators (Amo et al. 2008, Roth et al. 2008), and the blue tit *Cyanistes caeruleus* uses olfactory cues to find and maintain aromatic material for nests (Petit et al. 2002). Furthermore, in an examination of olfactory receptor (OR) genes across several avian species, Steiger et al. (2008) found that in all species included in their study, the majority of the genes amplified were functional genes, not pseudogenes. Interestingly, the estimated total number of OR genes for a passerine, the blue tit, was comparable to the number estimated for the snow petrel *Pagodroma nivea*, a Procellariiform with one of the largest olfactory bulb-to-brain ratios reported (Bang and Cobb 1968): 218 vs. 212 estimated OR genes, respectively (Steiger et al. 2008).

Few studies have investigated the role of olfaction in parent-offspring recognition in birds. The importance of odor in mother-offspring recognition and its effects on maternal behavior in many mammalian species are well documented (e.g., Lévy et al. 2004, Brennan and Kendrick 2006). Some evidence suggests that maternal behavior is affected not only by odors produced by the young themselves but by the presence of the mother's own odor on the offspring; for example, female rabbits may reject their own offspring if the offspring have been marked with the odor of another female (Mykytowycz 1968). Similarly, ring doves *Streptopelia risoria* were found to reject squabs who had been treated with an alien odor, suggesting that offspring odor may also be important in avian parental care (Cohen 1981). The acceptance of brood parasite young such as brown-headed cowbirds *Molothrus ater* by host species suggests that if odor plays a role in avian mother-offspring recognition, then the odor may actually be that of the incubating or brooding female herself. One possible source of this odor is preen oil from the uropygial gland (Soini et al. 2007), which is rubbed onto the feathers by birds while preening (though some birds with plumage odor lack odored preen gland secretions: Hagelin and Jones 2007). In this study, we test whether female songbirds reduce the length of incubation when preen oil from conspecifics or heterospecifics is applied to the nest

and eggs, as compared to when the female's own preen oil is applied.

The dark-eyed junco *Junco hyemalis* is a territorial, socially monogamous songbird species. Males do not build nests or incubate eggs (Nolan et al. 2002). Thus, the only avian odor on the eggs should be from the incubating female herself. Incubation involves long periods of sitting and warming the eggs with the body. No feces accumulate at the nest, so any odor imparted from the female is likely to be from preen oil rubbed off from her feathers. In junco preen oil, volatile compounds such as linear alcohols increase significantly during the breeding season, suggesting that preen oil odors are strongest during this time. Sex differences, as well as a high degree of individual variation, in relative volatile compound concentration appear only during the breeding season (Soini et al. 2007).

We manipulated the odor of junco nests and examined the effect on incubation behavior. We treated nests with the preen oil of either: (A) a conspecific from another population (to avoid confounding the study by using closely related individuals who may have similar odors), (B) a member of another passerine species, the northern mockingbird *Mimus polyglottos*, (C) the female's own preen oil, as a control condition, or (D) a vehicle-only control (acetone). We predicted that if females recognized odors (A) and (B) as foreign, they would reduce incubation time. Birds whose nests were treated with condition (C) or (D) should show no change in incubation behavior. Furthermore, we predicted that females would have a stronger reaction to the odor of a heterospecific (B) than of an unfamiliar conspecific (A).

## Methods

### Study site

Our research group has studied dark-eyed juncos at Mountain Lake Biological Station (MLBS) in Virginia (37° 22'N, 80° 32'W) continuously since 1983. From Apr. 15 to May 15, we catch juncos passively using mist nets and walk-in traps to census returning individuals and to band new birds with USFWS bands. From May 15 to July 15, we intensively search for nests. Once found, we identify the adults associated with the nest, count the number of eggs laid, and monitor the nest every three days from laying to hatching to fledging.

### Preen oil collection

Preen oil was collected by gently prodding the uropygial gland with a 100  $\mu$ l glass capillary tube until a small amount of preen oil (1–2 mg) was expelled and collected in the tube (Soini et al. 2007). We collected eight preen oil samples from six captive juncos (four male, two female) held at Indiana Univ. (IU); these juncos were originally captured from wild populations in southern California and thus were not closely related to the MLBS juncos. Eight preen oil samples were also collected from three captive male mockingbirds at IU using the same method. For the "self" treatment, preen oil was collected from all female Mountain Lake juncos caught during the early season capture and

census of 2008. All three groups were held in a photoperiod simulating long days (~15 h light, 9 h dark) and thus brought into breeding condition (A, B), or were in breeding condition in their natural habitat (C) at the time of preen oil collection. All preen oil samples were frozen at -20° C at IU and at MLBS until used. Just prior to application to the nest and eggs, a preen oil solution was made by suspending ~1 mg of preen oil into 200  $\mu$ l of acetone; the concentration of preen oil in the solution was similar for all treatment groups. Acetone was used as a solvent and immersing agent for the preen oil. Low molecular weight acetone is much more volatile than any of the preen oil compounds and therefore a short time after exposing the mixture to the air, the only remaining components on the nest/egg surfaces are the preen oil compounds. Furthermore, acetone is not expected to react with individual preen oil compounds (H. Soini pers. comm.).

Junco preen oil contains a number of volatiles, the most abundant being linear alcohols (C<sub>10</sub>–C<sub>18</sub>), methylketones, and carboxylic acids (Soini et al. 2007). Males and females differ in relative concentrations of some of these volatiles but in general have the same volatile compounds; one notable difference is that males have higher concentrations of the methylketones 2-tridecanone and 2-pentadecanone (Soini et al. 2007). Gas chromatography-mass spectrometry data suggest that preen oils from captive and wild juncos are very similar in volatile compound composition and relative concentration even though the diet is different (Whittaker et al. unpubl. data). Mockingbird preen oil also contains some of the same linear alcohols, but also has relatively high concentrations of branched alcohols such as 2-pentadecanol, 2-hexadecanol, and 2-octadecanol, which are absent in junco preen oil (Whittaker et al. unpubl. data). Measured volatile compound concentrations from all groups sampled in this study captive juncos, wild juncos, and captive mockingbirds—were all within comparable concentration ranges. Molecular weights of separate compounds varied also in the same range (120–320 Da) eluting within the retention time range of 10–60 min in the analytical conditions used. Therefore the volatility of preen oil compounds could be considered very similar in juncos and mockingbirds (Whittaker et al. unpubl. data).

### Treatment and collection of behavioral data

In the summer of 2008, experimental nests in the field were randomly assigned to one of three treatment groups: (A) conspecific (California junco, n = 8), (B) heterospecific (mockingbird, n = 8), or (C) self (n = 8). In the summer of 2009 we tested an additional set of nests with a fourth condition, (D) vehicle control (n = 6). No nesting female was used as a subject more than once. Each nest assigned to treatment groups (A) and (B) received preen oil solution from a single junco or mockingbird sample chosen at random. We used eight different samples from three different mockingbirds, and eight different samples from six juncos. Inspection of the data indicated that responses to all donors within a treatment group were similar, so we consider individual subjects as independent data points.

Juncos typically incubate eggs for 12 d before hatching. Most trials (n = 20) were conducted on nests that were on d

3–8 of incubation, but a few ( $n=6$ ) were in the later stages of incubation (d 9–11). A few nests were at an unknown incubation stage due to unknown laying date plus predation before hatching ( $n=4$ ). Post-hoc comparisons revealed no differences in the results due to incubation stage. We videotaped each nest for 4 h on the day before treatment to provide individual baseline data. On the second day, the preen oil solution (or pure acetone for condition D) was applied directly to an incubating female's eggs and nest using a pipetter. We applied 5  $\mu\text{l}$  of solution to each egg and 25  $\mu\text{l}$  (in five 5- $\mu\text{l}$  drops) to the edges of the nest. We then videotaped the nest for 4 h immediately following treatment. Both taping sessions were conducted at the same time each day (either 8 am – noon, or 1 pm – 5 pm). For each session, we flushed the female off the nest prior to setting up the video camera, and placed cameras 3–5 m from the nest. Data for conditions A, B, and C were collected over the period between May 5 and June 30 2008, and treatments were randomly assigned across that period of time; we collected data for the vehicle-only control in June–July 2009. To reduce inter-observer error and bias, all of the videos were watched by DJW in random order without reference to nest ID or treatment group. Each tape was scored for incubation start and end times.

### Analysis

We measured: 1) the length of the female's first incubation bout upon returning to the nest, 2) the average length of her incubation bouts, and 3) the proportion of time she spent on the nest. Average incubation bout length was found to correlate significantly with both first incubation bout length and proportion of time on nest ( $r=0.659$ ,  $P<0.001$ ), so it was removed from further analysis. We compared the

change in these measurements from the day prior to treatment to immediately after treatment using a one-way ANOVA in SPSS 16.0.

### Results

Only the change in the length of the first incubation bout differed significantly by odor. For females receiving the conspecific odor (treatment A,  $n=8$ ), the mean  $\pm$  SD change in the length of the first incubation bout was  $-0.18 \pm 0.19$  h; for the heterospecific odor (treatment B,  $n=8$ ) mean change was  $-0.40 \pm 0.36$  h; for self odor (treatment C,  $n=8$ ) the change was  $-0.02 \pm 0.19$  h; and in the acetone-only control group (treatment D,  $n=6$ ), mean change was  $-0.06 \pm 0.21$  h (one-way ANOVA,  $P=0.03$ ). The difference was most pronounced in the heterospecific treatment (B, Fig. 1). Fisher's LSD post-hoc tests revealed that the only significant pairwise difference was between responses to the self and mockingbird odors ( $P=0.006$ ) and between acetone and mockingbird odor ( $P=0.023$ ); response to conspecific odor did not significantly differ from heterospecific ( $P=0.105$ ), self ( $P=0.197$ ), or acetone ( $P=0.393$ ) treatments. Response to one's own preen oil did not differ from response to acetone only ( $P=0.724$ ).

No difference was observed among the treatment groups in proportion of total time spent on the nest (means  $\pm$  SD: (A) pre-treatment,  $0.79 \pm 0.07$ , post-treatment,  $0.77 \pm 0.06$ ; (B) pre-treatment,  $0.78 \pm 0.07$ , post-treatment,  $0.75 \pm 0.12$ ; (C) pre-treatment,  $0.68 \pm 0.21$ , post-treatment,  $0.78 \pm 0.13$ ; (D) pre-treatment,  $0.76 \pm 0.10$ , post-treatment,  $0.80 \pm 0.05$ ; one-way ANOVA,  $P=0.255$ ), suggesting that any effect of the preen oil treatment on behavior was temporary and any loss of incubation time was compensated for later in the trial. Furthermore, the

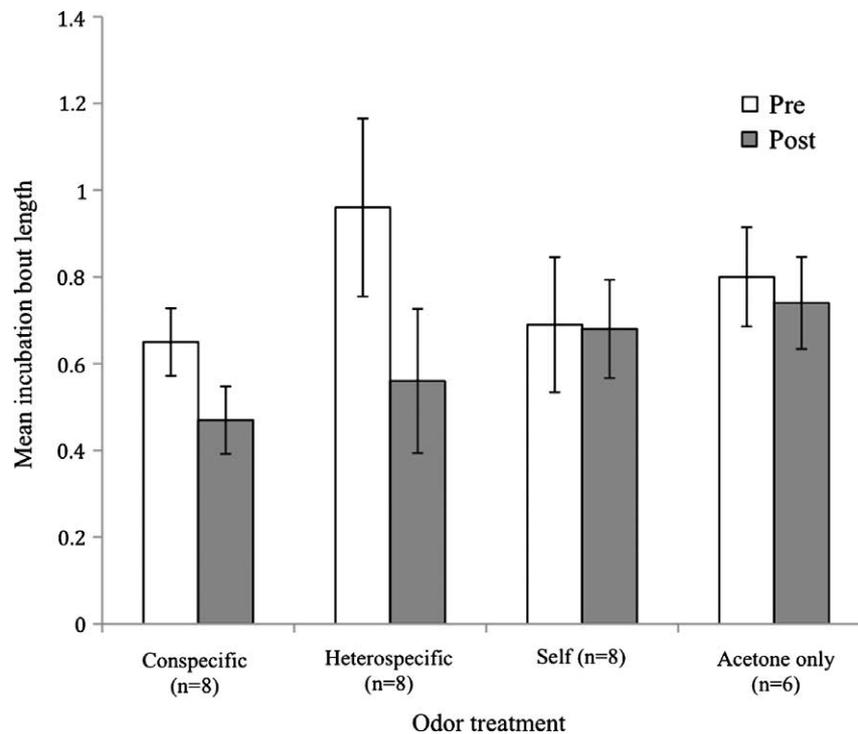


Figure 1. Mean  $\pm$  SEM first incubation bout length (h) before and after treatment.

temporary change in behavior had no effect on hatching success (proportion of eggs hatched in nests where at least one egg hatched, mean  $\pm$  SD: (A)  $0.91 \pm 0.16$ ; (B)  $1.0 \pm 0.0$ ; (C)  $0.92 \pm 0.20$ ; (D)  $1.0 \pm 0.0$ ; one-way ANOVA,  $P = 0.47$ ).

As a post-hoc question, we also looked at whether the sex of the preen oil donor used in the conspecific treatment had an effect on behavior. Nesting females appeared to have a stronger reaction to preen oil from male juncos (mean  $\pm$  SD reduction in first incubation bout length,  $-0.265 \pm 0.19$  h,  $n = 5$ ) than to preen oil from female juncos ( $-0.047 \pm 0.19$  h,  $n = 3$ ). The difference is not significant (one-way ANOVA,  $P = 0.141$ ); however, sample sizes are extremely small.

## Discussion

As predicted, we observed a significant effect of odor on female incubation bout length. This difference was observed only in the first incubation bout after returning to the nest at the beginning of data collection, when the treatment odor would be strongest and the female did not yet have the opportunity to become habituated. A similar effect was noted in the hesitation of blue tits when entering nests with experimentally manipulated plant odors, suggesting that birds may become quickly habituated to foreign odors (Mennerat 2008).

Incubating female birds put their heads and bills into nests to move and turn eggs, leaving open the possibility that they became aware of the application of alien secretions via vision or taste. However, we find these alternative explanations unlikely; female juncos rarely pause to inspect the nest before entering and instead begin incubating immediately upon arrival. Furthermore, in at least four nests observed, the females did not put their heads into the nest at all during the first incubation bout. We suggest that olfaction is the simplest explanation for the observed change in behavior.

The strongest effect observed in our study was the reduction of incubation time in response to heterospecific preen oil. Mockingbird preen oil contains volatile compounds distinct from those found in junco preen oil, and the juncos in our study may have detected this difference in odor at the nest. Evidence of the presence of heterospecifics in one's nest likely suggests a threat to one's reproductive success: many songbirds are affected by brood parasites or by nest site usurpers (e.g., house sparrows *Passer domesticus*). However, juncos (and many other species) do raise the offspring of brood parasites, thus a stronger reaction than observed in this study, such as ejecting eggs or abandoning the nest, would be unlikely. Cowbirds often lay a single egg in a junco's nest, and often remove some of the junco's eggs; however, in nearly half of parasitized nests (and often more, pers. obs.), the junco's own eggs are left intact (Wolf 1987). Though parasitized juncos fledge fewer young per nest than unparasitized juncos, the proportion of hatched eggs that successfully fledge is similar in both groups (Wolf 1987). Thus, juncos are typically able to gain some reproductive success from parasitized broods, and it may be non-adaptive to abandon parasitized nests and expend the energy required to begin a new brood. On the other hand, nest desertion in

response to parasitism is more common in some other passerine species (e.g., Goguen and Mathews 1996), and in those species, application of alien secretions might stimulate desertion; conversely, adding the nesting female's own preen oil to eggs of brood parasites might prevent desertion.

The response to conspecific secretions was intermediate between the responses to heterospecific and to one's own secretions, and, based on the analyses presented here, was not significantly different from either. However, interpretation of these results is complicated by the fact that secretions from different sexes were used (only male heterospecifics, but male and female conspecifics). The response to female conspecifics was much closer to the response to a female's own preen oil, while the response to male conspecifics was closer to the response to heterospecifics. As noted earlier, there are some sex differences in male and female junco preen oil, with higher concentrations of 2-tridecanone and 2-pentadecanone in male preen oil (Soini et al. 2007). Future studies must control for sex of the preen oil donor, and should examine the ability of juncos to differentiate among odors based on sex.

The only previous study to examine the effect of odor on avian parental care had more extreme results, with some parents rejecting nestlings that had been treated with alien odors (Cohen 1981). However, that study was conducted in captivity and used odors that would not normally be encountered (citrus odors), while the present study attempted to simulate odors that may be left behind if another bird enters the nest. We observed a measurable but temporary effect, suggesting that juncos do sense odors from both conspecific and heterospecific preen oil, but that this stimulus was not sufficient to change parental behavior. However, our observation of reactions to heterospecific and male conspecific preen oils has interesting implications for future research about the role of odor transmitted via preen oil in passerine reproductive behavior.

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