INTRODUCTION

A central focus in evolutionary biology is to understand how species originate and how existing species boundaries are maintained. Natural hybridization—when separate species come into contact and mate—can provide important insights into the speciation process (Abbott et al., 2013). In animals, behavioral isolation (i.e., premating isolation) can act to maintain species boundaries upon secondary contact (reviewed in Servedio & Noor, 2003). Chemical communication in particular has been shown to act as an important premating isolating barrier in a wide variety of animal taxa (Smadja & Butlin, 2009) including insects (Coyne, Crittenden, & Mah, 1994; Sasakawa & Kon, 2018), fish (Kodric-Brown & Streeker, 2001; Kozak, Head, & Boughman, 2011), reptiles (Barbosa, Font, Desfilis, & Carretero, 2006), amphibians (Dawley, 1984), and mammals (Johnston, 2003). However, conspecific preferences for interspecific odor cues have never been documented in a natural songbird hybrid zone (Caro, Balthazart, & Bonadonna, 2015).

Songbird hybrid zones have provided many insights into how behavioral isolation maintains species boundaries upon secondary contact (Edwards et al., 2005). While visual signals (Bleiweiss, 2004; Moller & Cuervo, 1998; Patten, Rotenberry, & Zuk, 2004; Sætre et al., 1997) and auditory signals (Haavie et al., 2004; Irwin, 2000; King, West, & Eastzer, 1980; Patten et al., 2004;
Slabbekoorn & Smith, 2002) have been shown to act as premating barriers between hybridizing songbird species, such a role for olfaction has not been documented. However, songbirds do possess a working olfactory system (reviewed in Balthazart & Taziaux, 2010), including olfactory bulbs (Bang & Cobb, 1968), olfactory receptors (Steiger, Fidler, Valcu, & Kempenaers, 2008; Steiger, Kuryshov, Stensmyr, Kempenaers, & Mueller, 2009), and the ability to detect odor cues (Clark, Avilova, & Bean, 1993). Olfactory information for songbirds is most likely carried in uropygial gland oils, which have been shown to possess species-specific chemical compositions (Soini, Whittaker, Wiesler, Ketterson, & Novotny, 2013). Recent work suggests a role for olfaction in many aspects of avian ecology (reviewed in Caro & Balthazart, 2012) including species discrimination (Bonnadonna & Mardon, 2010; Whittaker et al., 2011; Zhang, Du, & Zhang, 2013), sex discrimination (Amo et al., 2012; Soini et al., 2007; Whittaker et al., 2010; Zhang, Sun, & Zuo, 2009; Zhang, Wei, Zhang, & Yang, 2010), threat detection (Amo, Galván, Tomás, & Sanz, 2008; Amo, Visser, & Oers, 2011; Roth, Cox, & Lima, 2008), individual quality (Amo et al., 2012; Whittaker, Gerlach, Soini, Novotny, & Kettersson, 2013), aggression (Whittaker et al., 2018), nest recognition (Caspers, Hoffman, Kohlmeier, Krüger, & Krause, 2013; Golüke, Dörrenberg, Krause, & Caspers, 2016), and kin recognition (Bonadonna & Sanz-Aguilar, 2012; Caspers, Gagliardo, & Krause, 2015; Caspers et al., 2017; Coffin, Watters, & Mateo, 2011; Krause, Kruger, Kohlmeier, & Caspers, 2012). Still, whether conspecific preferences for divergent odor cues exist in natural songbird hybrid zones and therefore whether odor cues might function as a premating barrier in songbirds remain unknown (Campagna, Mardon, Celerier, & Bonadonna, 2011; Caro et al., 2015). Here, we explore the potential for olfactory communication to act in premating reproductive isolation between the black-capped and Carolina chickadee.

The black-capped (Poecile atricapillus) and Carolina chickadee (P. carolinensis) are sister taxa (Harris, Carling, & Lovette, 2014) that occupy parapatric ranges in North America. The black-capped chickadee resides in the northern half of the United States and in the southern half of Canada, while the Carolina chickadee occupies a range in the southeastern United States (Figure 1; McQuillan & Rice, 2015). The ranges of these two species overlap in a long but very narrow hybrid zone that stretches from New Jersey to Kansas, which is moving northward due to climate change (Figure 1; Taylor, Curry, White, Ferretti, & Lovette, 2014; Taylor, White, et al., 2014). Within this region of sympathy, the two species are able to successfully hybridize. However, postzygotic reproductive barriers are present. Similar to findings from other parts of the hybrid zone (Bronson, Grubb, Sattler, & Braun, 2005), breeding data collected from our hybrid zone transect in southeastern Pennsylvania shows that eggs produced by mixed-species parental pairs are less likely to hatch compared to eggs produced by conspecific pairings (Figure S1). Additional costs to hybridization are also found in adult birds of mixed ancestry. Hybrid chickadees are compromised in their spatial memory ability (McQuillan, Roth, Huynh, & Rice, 2018), which is important for fitness in scatter-hoarding species such as chickadees (Sonnenberg, Branch, Pitera, Bridge, & Pravosudov, 2019). Hybrid chickadees also are less likely to solve novel problems (McQuillan et al., 2018). Taken together, the reduced hatching success of hybrid offspring combined with cognitive deficiencies in adult hybrids suggests there are severe costs to hybridization for chickadees in our eastern Pennsylvania hybrid zone populations. Further, within the hybrid zone, the black-capped and Carolina chickadee possess similar plumage (Robbins, Braun, & Tobey, 1986) and each species can learn the song of the other (Kroodsma, Albano, Houlihan, & Wells, 1995). Therefore, these two signals commonly implicated in behavioral isolation in songbirds may not be reliable indicators of species
identity in this chickadee hybrid zone. We therefore sought to test two requirements for olfactory signaling to function in premating reproductive isolation between currently hybridizing black-capped and Carolina chickadees: (1) the production of species-specific chemical compounds and (2) conspecific odor preferences.

Here, we report chemical differences in wild-caught hybrid zone black-capped and Carolina chickadee uropygial oils. Uropygial oils are a main source of odor cues in birds, the chemical composition of which is known to be species-specific across many songbird species (Soini et al., 2013). We also show that hybrid zone birds of both species show preferences for conspecific whole-body odor cues over heterospecific whole-body odor cues, suggesting a possible role for olfaction in reproductive isolation.

2 | METHODS

2.1 | Field methods and animal housing

We captured wild chickadees at five geographically proximate sites within the hybrid zone in eastern Pennsylvania (total transect length 30 km; Nockamixon State Park 40°26’10.7″N, 75°14’39.0″W, DeSales University 40°32’41.7″N, 75°22’29.5″W, Lehigh University 40°36’5.2″N, 75°21’34.1″W; Jacobsburg State Park 40°47’4.0″N, 75°17’34.7″W; Bangor 40°53’42.8″N, 75°10’32.0″W). Birds were caught using mist nets at feeders or by using song playback in conjunction with a clay chickadee model. Upon capture, we banded, measured, and weighed each bird. A small blood sample was collected for ancestry (McQuillan, Huynh, Taylor, & Rice, 2017) and sex determination (Griffiths, Double, Orr, & Dawson, 1998). All procedures were approved by Lehigh University’s Institutional Animal Care and Use Committee (Protocol #215). Animal capture and transport were conducted under the U.S. Geological Survey federal bird banding permit 23810, U.S. Fish and Wildlife Service permit MB9567A-0, Pennsylvania Game Commission permits 103 and 145, and Pennsylvania Bureau of State Parks permit 2016–18.

For the chemical analysis of uropygial oils, we sampled 41 black-capped and 40 Carolina adult chickadees between December 2015 and December 2016. The uropygial gland of these birds was cleaned with 75% ethanol and gently squeezed with sterile forceps. A small sample of oil (~1 mg) was collected in the tip of a capillary tube. Sampled oils were dried with 75% ethanol and gently squeezed with sterile forceps. A small sample of oil (~1 mg) was collected in the tip of a capillary tube. For the behavioral tests of odor preference, we captured 20 black-capped males, 9 black-capped females, 20 Carolina males, and 10 Carolina females between February 2017 and November 2018 and transported them by car to an outdoor aviary at Lehigh University. The difference in capture rate between the sexes could be due to a number of factors, including sex differences in attraction to our model and song playback. No birds were tested or held in captivity during the breeding season (mid-March through July). Birds were housed individually in 0.46 m × 0.61 m × 0.61 m cages during their testing period and were visually but not aurally isolated from one another. During this time, all birds were sustained on an ad libitum diet of sunflower seeds, pine nuts, and water containing a vitamin supplement, as well as 15 waxworms and 20 mealworms per day.

2.2 | Genetic determination of species ancestry

Because black-capped, Carolina, and hybrid chickadees are morphologically similar and song is not a reliable species identifier within the hybrid zone (Kroodsma et al., 1995), we utilized genetic markers to assign ancestry to each bird (McQuillan et al., 2017, 2018). Briefly, genomic DNA was extracted from blood samples using a Qiagen DNeasy blood and tissue kit (QIAGEN). We genotyped all birds at 10 species-diagnostic single nucleotide polymorphism markers (McQuillan et al., 2017). We used STRUCTURE (Hubisz, Falush, Stephens, & Pritchard, 2009) to estimate admixture proportions and assign ancestry categories for each bird. To do this, we combined the genotypes of our test subjects with a larger dataset from multiple Pennsylvania hybrid-zone populations, as well as known pure-species individuals from allopatric populations of both species (New York and Louisiana, USA). We ran STRUCTURE on this larger dataset of over 400 total genotypes using the same program settings as McQuillan et al. (2017, 2018). Following McQuillan et al. (2018), birds with admixture values within the average 90% credible interval of known pure individuals were classified as either a pure black-capped or Carolina individual. In contrast, birds with admixture values outside of the average 90% credible interval for known parentals were classified as hybrids and were not used in this study.

2.3 | Uropygial oil collection and GC-MS

Oil samples were extracted in 200 μl of dichloromethane overnight at 4°C. Chemical analysis was performed using gas chromatography–mass spectrometry (Shimadzu QP 2010 Ultra GC-MS equipped with a SHRX1-5US column; 30 m length, 0.25 mm thickness). Samples were run using conditions adapted from Zhang et al. (2013). Runs were performed in splitless mode on 3 μl of sample at a linear ramp of 70°C to 280°C over 42 min with a final hold at 280°C for 15 min (column pressure, 31.6 psi; total flow, 101.3 μl/min; column flow 3.86 ml/min; linear velocity 72 cm/s). We measured the relative abundance or total proportion of each compound by integrating the area under each peak and converting this area into a percentage of the total gas chromatogram area.

2.4 | Odor preference trials

We tested males and females of both species for their odor preferences. We chose to test preferences in both sexes because in species showing biparental care, as is the case in chickadees, both female and male preferences have been shown to be evolutionarily significant (Edward & Chapman, 2011). All birds were subjected to two sequential no-choice preference trials in a Y-maze chamber (Figure 2; height: 20 cm, choice arms: 45 cm, starting arm: 30 cm, width of choice arms and starting arm: 20 cm). While both sequential no-choice trials and two-choice trials allow a measure of which odor individuals prefer (Dougherty & Shuker, 2015), we used no-choice trials because they additionally provide information on the absolute preference of each odor individually. Absolute preferences for...
to the center of the Y-maze. All testing apparatuses were cleaned with ethanol and allowed to air dry between trials.

In our Y-maze design, we used live birds as odor sources; we therefore tested preferences for whole-body odor cues as opposed to odors from only uropygial oil secretions. The chemical composition of uropygial oil secretions and chemicals extracted directly from feathers have been shown to differ (Sandilands, Powell, Keeling, & Savory, 2004; Zhang et al., 2013). Results from whole-body odor preference tests may be more ecologically relevant to how these birds encounter odor cues in the wild, as this method incorporates all odors and not just those derived from the uropygial gland.

2.5 | Statistical analysis

2.5.1 | Uropygial oils

To correct for the nonindependence of our proportion data, we used an empirical log transformation by taking the natural logarithm of

\[ p + \varepsilon/(1-p + \varepsilon), \]

where \( p \) is the proportion of that compound in the entire sample and \( \varepsilon \) is the minimum nonzero proportion of the dataset, that is, 0.01 (Amo et al., 2012; Armitage, Berry, & Matthews, 1994; Baum, 2008; Warton & Hui, 2011; Whittaker et al., 2010; Zhang et al., 2013). To test for differences between black-capped and Carolina chickadees in uropygial oil chemical profiles, we first conducted a principal components analysis (PCA) on the transformed gas chromatograph data (N = 81) to reduce the dataset of 146 different compounds into a smaller number of principal components (PCs). PCA is the most common method used to analyze avian preen oil chemistry (Amo et al., 2012; Fischer, Haliński, Meissner, Stepnowski, & Knitter, 2017; Leclaire et al., 2012; Lopez-Perea & Mateo, 2019; Shaw, Rutter, Austin, Garvin, & Whelan, 2011; Tuttle et al., 2014; Whittaker et al., 2013; Whittaker et al., 2019; Whittaker et al., 2018; Whittaker et al., 2010; Zhang et al., 2013) and it allows an examination of which factors best explain the main axes of variation in our data, represented by PCs. We used a multivariate analysis of variance (MANOVA) on nontrivial principal components selected based on comparing a scree plot of the principal components with a broken-stick distribution (Jackson, 1993), using species, date, sex, and all interactions as fixed factors.

We then used type-II ANOVAs and least square means comparisons to further analyze individual PCs. Examining individual PCs in this way allowed us to look for species differences, while controlling for the effects of sampling date and the potential interaction effects of sampling date with other factors. This is particularly important concerning our data, since we collected preen oil samples throughout the calendar year and significant seasonal variation in preen oil chemistry has been observed in other species (Reneerkens, Piersma, & Damsté, 2002, 2005; Soini et al., 2007; Whelan, Levin, Owen, & Garvin 2010; Whittaker et al., 2019).

To analyze models of each individual PC, we performed a stepwise model simplification procedure by removing the least significant variable, starting with higher order interactions; the initial full model contained species, date, sex, and all interactions as fixed
TABLE 1  Model selection based on LRT and AICc for PC1, PC2, and the ratio of ester to nonester compounds

| Model                           | LRT    | AICc   |
|---------------------------------|--------|--------|
| **PC1**                         |        |        |
| Full model                      | 365.2  | 359.9  |
| Species, date                   | .379   | 357.8  |
| Species                         | .631   | 357.8  |
| Species × date                  | <.001  | 393.2  |
| Date                            | .046   | 359.8  |
| **PC2**                         |        |        |
| Full model                      | 321.1  | 327.2  |
| Species, date, sex, species × date, species × sex, date × sex | .004 | 832.7 |
| **Ratio of ester to nonester compounds** |        |        |
| Full model                      | 831.0  |        |
| Species, date, sex, species × date, species × sex, date × sex | .119 | 831.1 |
| Species, date, sex, species × date, species × sex | .002 | 838.7 |
| Species, date, sex, species × date, date × sex | .033 | 833.4 |
| Species, date, sex, species × sex, date × sex | .924 | 828.5 |

Note: LRT p-values are for the specified model compared to the last model above it that passed the LRT (p-value > .05). Best-fit models are highlighted in bold.

2.5.2 | Odor preference trials

Because of the small sample sizes of each group (black-capped male \( n = 19 \); black-capped female \( n = 9 \); Carolina male \( n = 17 \); Carolina female \( n = 10 \)), we tested the hypothesis that conspecific odors are preferred over heterospecific odors by using paired one-tailed nonparametric Wilcoxon rank-sum tests to compare time spent by chickadees with conspecific versus heterospecific odors. We also tested whether odor preferences differed from the random expectation for time spent in the choice arm of the Y-maze (ratio of choice area volume/total Y-maze volume × test duration of 900 s (252 s)) using one-sample Wilcoxon rank-sum tests. To test whether the strength of conspecific odor preferences varied throughout the year, we fit a linear model fit of the time spent with the conspecific odor by the fixed factors of Julian day, species, and sex. All analyses were conducted in R [3.5.2] (R Core Team, 2018).

3 | RESULTS

3.1 | Uropygial oils

The gas chromatograms of our chickadee oil samples revealed on average 34 (±22) compounds in a single individual’s oil sample. Compound class identification using a NIST08 mass spec library predicted linear and branched alkanes eluting prior to ~35 min and ester compounds eluting after ~35 min.

We selected the first 4 principal components based on the scree plot of our analysis (Figure S2), which together explained 64% of the variation in the total relative abundances of all compounds (35%, 14%, 8%, and 7%, respectively). These principal components differed significantly by species (MANOVA, \( F = 10.41, p < .001 \)) and by date (MANOVA, \( F = 43.66, p < .001 \)). We also found a significant interaction between species and date (MANOVA, \( F = 2.64, p < .05 \)). While the two sexes did not differ significantly in PC values (MANOVA, \( F = 0.81, p = .52 \)), there was a significant three-way interaction of species, date, and sex (MANOVA, \( F = 2.91, p < .05 \)).

Our best-fit model for PC1 contained only species and date as fixed factors (Table 1). PC1 values were significantly different between the two species and varied by date (Table 2), with black-capped chickadees showing significantly higher PC1 values than Carolina chickadees (LMMeans contrast t-ratio = 2.00, \( p < .05 \)). Based on its rotation values, PC1 was negatively correlated with earlier eluting alkane compounds and positively correlated with later eluting ester compounds (Table S1). Our best-fit model for the ratio of ester to nonester compounds included the three main effects of species, date, and sex as well as the interaction term.
of species × sex and date × sex (Table 1). Corresponding with the species differences in PC1, the ratio of ester to nonester compounds was significantly greater in black-capped chickadees than in Carolina chickadees (Table 2, LSmeans contrast \( t \)-ratio = 3.13, \( p < .01 \)). Although the effect of species changes depending on sex as indicated by the significant species × sex two-way interaction (Table 2), a plot of the interaction shows that it does not preclude our ability to interpret the simple main effect of species (Figure S3). Our best-fit model for PC2 was the full model with species, date, sex, and all interactions as fixed factors (Table 1). PC2 showed a more complicated relationship with a significant main effect of species, significant two-way interactions of species × date and species × sex, as well as a significant three-way interaction of species, date, and sex (Table 2). As shown by an interaction plot, the significant influence of sex on species differences did not preclude our ability to interpret the simple main effect of species (Figure S4). Nominalizing date to before, during, and after the breeding season revealed species differences in PC2 during the breeding season period (Figure S5). Because of this interaction, we restricted our analysis of PC2 to individuals caught only during the breeding season and found that black-capped chickadees have significantly higher PC2 values (LSmeans contrast \( t \)-ratio = 3.31, \( p < .01 \)). However, the two species still show a similar significant difference in PC2 even without this restriction (LSmeans contrast \( t \)-ratio = 3.43, \( p < .01 \)). There were no significant species differences in PCs 3 and 4.

### 3.1.1 Odor preference trials

Male birds of both species showed significant preferences for conspecific female odors over heterospecific female odors (Figure 3a; black-capped male \( V = 163, p < .01 \); Carolina male \( V = 132, p < .01 \)). Time spent by males with conspecific female odor was significantly higher than expected for random movement (Figure 3a; black-capped male \( V = 180, p < .001 \); Carolina male \( V = 136, p < .01 \)), while the time spent with heterospecific female odor was not different from random movement (Figure 3a; black-capped male \( V = 102, p = .8 \); Carolina male \( V = 88, p = .6 \)). Likewise, both groups of female birds also showed significant

### TABLE 2

Analysis of variance (type-II tests) of PC1 (Adjusted \( R^2 = .41, F_{2,78} = 28.37, p < .001 \)), PC2 (Adjusted \( R^2 = .27, F_{7,64} = 4.737, p < .001 \)), and the ratio of ester to nonester compounds (Adjusted \( R^2 = .23, F_{5,66} = 5.32, p < .001 \))

| SS  | df  | F-value | p-Value |
|-----|-----|---------|---------|
| PC1 |     |         |         |
| Species | 32.34 | 1 | 3.991 | .049 |
| Date | 420.53 | 1 | 51.907 | <.001 |
| Residual | 631.92 | 78 |
| PC2 |     |         |         |
| Species | 28.266 | 2 | 3.319 | .043 |
| Date | 0.196 | 1 | 0.046 | .831 |
| Species × date | 60.929 | 1 | 14.308 | <.001 |
| Species × sex | 19.192 | 1 | 4.507 | .038 |
| Date × sex | 12.611 | 1 | 2.961 | .009 |
| Species × date × sex | 35.309 | 1 | 8.291 | .005 |
| Residual | 272.545 | 64 |
| Ratio of ester to nonester compounds |     |         |         |
| Species | 56,367 | 1 | 11.043 | .001 |
| Date | 50,151 | 1 | 9.825 | .003 |
| Sex | 2,773 | 1 | 0.543 | .464 |
| Species × sex | 25,935 | 1 | 5.081 | .028 |
| Date × sex | 55,554 | 1 | 10.884 | .002 |
| Residual | 336,883 | 66 |

Note: Significant \( p \)-values are highlighted in bold.
preferences for conspecific male odors over heterospecific female odors (Figure 3b; black-capped female V = 39, p < .05; Carolina female V = 47, p < .05). Carolina females spent significantly more time with conspecific odors than expected for random movement (Figure 3b; V = 51, p < .05), but not with heterospecific odors (Figure 3b; V = 34, p = .5). However, black-capped female preferences for either male odor did not differ significantly from random movement (Figure 3b; for black-capped male odor V = 34.5, p = .17; for Carolina male odor V = 16, p = .5). Our data showed no apparent seasonal effect on odor preferences throughout the year in both sexes of either species (Figure S6).

4 | DISCUSSION

Overall, our results are consistent with a possible role for olfactory signaling in premating reproductive isolation in chickadees. Within the hybrid zone, these two species show differences in their uropygial oil chemistry (Table 2) as well as significant preferences for conspecific bird odors over those of heterospecific birds (Figure 3). To our knowledge, this is the first evidence of olfaction-based species discrimination in a natural songbird hybrid zone.

Reproductive isolation due to divergent olfactory signals and preferences is known to occur in insects (Löfstedt et al., 1986; Sasakawa & Kon, 2018; Singer, 1998), as well as in many vertebrate taxa including fish (Kozak et al., 2011; McLennan & Ryan, 1999; Rafferty & Boughman, 2006), amphibians (Dawley, 1984), reptiles (Barbosa et al., 2006), and mammals (Christophe & Baudoin, 1998). While discrimination between conspecific and heterospecific odors has been observed in several songbird systems, conspecific odor preference within naturally hybridizing songbird species has not been previously documented. For example, female waxwings were found to prefer odor cues of their own species over cues of their sympatric sibling species. However, because these two species do not currently engage in hybridization, it is unclear whether these preferences played a role in the actual speciation process (Zhang et al., 2013). Likewise, crimson rosellas have been found to discriminate between odors of related subspecies based on the latency for birds to enter their nest boxes when presented with odor cues (Mihailova, Berg, Buchanan, & Bennett, 2014), but these experiments did not test birds from their natural hybrid zone and direct preferences for odor cues were not measured. Additionally, while odor discrimination was found in juncos (Whittaker et al., 2011) and zebra finches (Krause et al., 2014), the heterospecifics used in these preference trials do not naturally hybridize with the focal species.

We found significant species differences in the principal component description of uropygial oil profiles of black-capped and Carolina chickadees, including differences in the ratio of wax-ester to nonester compounds (Table 2). Similar relative abundance ratios of compound mixtures are enough to encode communicatory information in mammals (Sun & Muller-Schwarze, 1998a, 1998b), insects (Byers & Struble, 1990; Coyne et al., 1994; Singer, 1998; Wang, Zhao, & Wang, 2005), and birds (Zhang et al., 2013) and shifts in wax-ester ratios have been found to coordinate with breeding in the Scolopacidae (Reneerkens, Piersma, & Damsté, 2002, 2005). Further, a number of studies show support for a genetic basis of uropygial oil chemistry in birds (Leclaire et al., 2012; Soini et al., 2013; Whittaker et al., 2010). Thus, chemical differences in songbird uropygial oils such as those found here could function in mate choice and reproductive isolation by carrying information on species identity.

Although little is known about the biosynthesis of uropygial oils, hormone levels (Whittaker et al., 2018), diet (Apandi & Edwards, 1964), and age (Sandilands et al., 2004) have all been shown to have effects on oil composition. Promising avenues of future research could address the possibility of uropygial oils acting as an honest signal of mate quality (McGlothlin et al., 2008; Velando, Beamonte-Barrientos, & Torres, 2006). For example, oil composition could carry information on hormone levels, which are known to be closely tied to cognitive ability (reviewed in Healy & Hurly, 2004) and aggression (Whittaker et al., 2018), both of which are important for fitness in chickadees (Bronson, Grubb, Sattler, & Braun, 2003; Sonnenberg et al., 2019). Additionally, whether or not these cues could potentially act as a “magic trait” during speciation with gene flow could be considered. Magic traits are those that are under divergent selection while also promoting assortative mating in sympathy (Servadio, Doorn, Kopp, Frame, & Nosil, 2011). Well-studied examples of magic traits include body size (Nagel & Schluter, 1998), body shape (Langerhans, Gifford, & Joseph, 2007), beak morphology and song (Podos, 2001), coloration (Reynolds & Fitzpatrick, 2007), and diet (Snowberg & Bolnick, 2008). If uropygial oil biosynthesis is affected by diet, then these oil-derived odor cues could serve as an indicator of resource specialization, potentially contributing to assortative mating within populations undergoing divergent adaptation. Further research on the factors affecting the chemical composition of uropygial oils, and on the role of oil-derived odor cues in the mate preferences of songbird populations in the early stages of divergence will be necessary to evaluate these possibilities.

Our experiments indicate a clear preference for conspecific whole-body odors in both species of chickadees (Figure 3). These preferences were present in male as well as female birds. Male mating preferences, especially in species displaying biparental care, can be just as important as female choice (Johnstone, Reynolds, & Deutsch, 1996) and recent work has begun to acknowledge the potentially widespread role of male mate choice in animals (reviewed in Edward & Chapman, 2011). Male choice can act during the selection of a partner (Jones, Monaghan, & Nager, 2001), or postcopulation, such as in the allocation of parental care after males have the opportunity to assess female quality (Matessi, Carmagnani, Griggio, & Pilastro, 2008). Further, mate preference models demonstrate that the evolution of reproductive isolation and reinforcement can occur solely through male mating preferences (Servadio, 2007). Male choice preference for female odor cues have been empirically shown in red-spotted newts (Verrell, 1985), spiders (Gaskett, Herberstein, Downes, & Elgar, 2004), and more recently in Drosophila, where such preferences drive reproductive isolation (Shahande, Pischedda, & Turner, 2017). Our odor preference results are consistent with the possible role of male mate choice in reproductive isolation.
In our tests, time spent with heterospecifics did not differ from random expectation in all four groups of birds. This raises the possibility that selection could be acting to promote conspecific preferences instead of avoidance to heterospecific odors. Hybridization, although costly, could incur fitness advantages if a conspecific mate cannot be found, in which case, aversion to heterospecific odors could be maladaptive. Interestingly, female black-capped chickadee preferences for conspecifics also did not differ from random expectation (Figure 3). This could indicate that odor cues are weighed differently between the sexes or species or that females may be incorporating additional types of information in their mate choice decisions. Bronson et al. (2003) found that both black-capped and Carolina females show preferences for black-capped males, but that this preference switches to Carolina males when they are allowed to observe male–male social interactions. Thus, mate choice may be more complex and context-dependent in female chickadees. Research on mating systems indicates that multiple cues may act in songbird mate choice (Bro-Jørgensen, 2009; Byers & Kroodsma, 2009; Candolin, 2003; Gil & Gahr, 2002; Hill, 2006; Otter & Ratcliffe, 1996; Patten et al., 2004; Whittaker & Gerlach, 2016). Emerging work contends that female birds show individual variation in their ability to perceive different modes of communication and that multimodal signaling is thus particularly important for advertising in males (Ronald, Fernández-Juricic, & Lucas, 2018). We suggest that odor could be an overlooked signal that contributes in a multimodal fashion with more traditionally studied mating cues such as song, plumage, and social rank in chickadees and in songbirds generally.

Odor cues may function directly as a mate choice cue or indirectly through their effects on other aspects of songbird ecology. chickadees overwinter in mixed-species flocks, during which time they can often be found in close proximity to one another and their pair bonds are formed prior to the start of the breeding season, in late winter into the early spring (Odum, 1941). While interacting in close proximity within these winter flocks, birds may be able to incorporate olfactory cues into their mate choice decisions. Although our Y-maze experimental design did not measure actual mate choice, which can differ from mate preferences depending on context (Yang, Blomenkamp, Dugas, Richards-Zawacki, & Pröhl, 2019), it did allow us to isolate and measure preferences solely for odor cues in wild-caught chickadees. Time spent with a potential mate or cue has been widely used as a proxy for mate choice (Bronson et al., 2003; Gaskett et al., 2004; Verrell, 1985; Yang, Richards-Zawacki, Devar, & Dugas, 2016), and similar Y-maze designs measuring time spent with odor cues have been used to assess odor preferences as a proxy for mate choice in songbirds (Bonadonna & Sanz-Aguilar, 2012; Whittaker et al., 2011; Zhang et al., 2013). While not addressed in our experiments, odor may also play indirect roles in chickadee mate choice, for example, through male–male social interactions. Social rank is important in mate choice in chickadees (Bronson et al., 2003), and uropygial oil composition has been found to correlate with aggression in other songbirds (Whittaker et al., 2018). Thus, how chemical signaling could influence other aspects of songbird behavior indirectly related to mate choice such as male–male interactions is an interesting avenue for future research.

Whether olfactory preferences in songbirds are generally learned or innate is unknown. Learned mating preferences are well documented, affecting sexual isolation and the speciation process (reviewed in Verzi~jen et al., 2012). In other taxa, prenatal chemosensory learning has been demonstrated (Caubet, Jaisson, & Lenoir, 1992; Courtenay, 1989; Hepper & Waldman, 1992; Schaal & Orgeur, 1992) and sexual imprinting on diet-based odor cues has been found to contribute to reproductive isolation (Delaney & Hoekstra, 2018; Kozak et al., 2011; Sasakawa & Kon, 2018). In several procellariform species, young chicks seem to demonstrate odor recognition (Cunningham, Buskirk, Bonadonna, Weimerskirch, & Nevitt, 2003; De Leon, Minguez, & Belliure, 2003). Preference for extraneous odors has been found to develop during the incubation period in chickens (Sneddon, Hadden, & Hepper, 1998) while preferences for parental odors may be determined earlier in egg development in zebra finches (Caspari et al., 2017). Within the same family as chickadees (Paridae), blue tits have also been shown to exhibit odor recognition at the nestling stage (Rossi et al., 2017). While more work needs to be done in this area, the possibility that odor preferences are formed during development in chickadees could facilitate its function in reproductive isolation if individuals learn preferences from parental odors in pure-species nests.

Although our results suggest that olfactory cues in this chickadee hybrid zone have the potential to contribute to assortative mating, it remains unknown whether direct selection against hybridization is driving the evolution of this pre-mating isolation (i.e., reinforcement; Lewontin, 1974; Servedio & Noor, 2003). Postzygotic isolation in this chickadee hybrid zone has been documented in the form of reduced hatching success of interspecific breeding pairs (Figure S1, Bronson et al., 2005) and in the reduced cognitive abilities of adult hybrids (McQuillan et al., 2018). To test whether this maladaptive hybridization is directly reinforcing uropygial oil differences and odor preferences within the hybrid zone, further work should be conducted in allopatric populations. Away from the hybrid zone, where hybridization cannot occur, a reduction in conspecific preferences and oil differences may be observed—that is, a pattern of reproductive character displacement (Pfennig & Pfennig, 2012). Chemical cues have been shown to undergo reinforcement across multiple taxa in natural hybrid zones (reviewed in Smadja & Butlin, 2009), but this has not yet been observed in an avian system.

Work in other systems raises the interesting possibility that hybridization could affect olfactory preferences in songbirds. Compromised olfactory ability has been found in hybrid insects (Olsson et al., 2006) and skewed olfactory preference in hybrid individuals has been shown in mice (Christophe & Baudoin, 1998). The odors and odor preferences of hybrid chickadees are unknown, yet these traits could influence the evolution of populations along their hybrid zone by affecting general patterns of mate choice and introgression. Alternatively, if hybrid chickadees are somehow compromised in these traits, this could contribute to postzygotic isolation through their inability to advertise successfully or assess mate quality.

In conclusion, our results highlight the role that uropygial oils and odor cues may be playing in songbird mate choice and pre-mating reproductive isolation along with more traditionally studied cues such as song
and plumage characteristics. The differences in oil profiles combined with conspecific preferences in both species of chickadees suggest that olfactory preferences, the possible reinforcement of these cues and preferences within the hybrid zone, and the chemical characteristics and preferences of hybrid individuals are promising avenues of future research.

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CONFLICT OF INTEREST

This paper is not under consideration for publication in any other journal. Both authors have read this manuscript, agreed to this submission, and have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

A.V.H. conceived of the project idea, performed experiments, and analyzed data. A.V.H. and A.M.R. designed experiments and wrote the manuscript.

DATA AVAILABILITY STATEMENT

All pertaining data files and R code can be found in the Dryad Digital Repository; https://doi.org/10.5061/dryad.7ht92p7.

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REFERENCES

Abbott, R., Albach, D., Ansell, S., Armsten, J. W., Baird, S. J. E., Bierne, N., ... Zimmer, D. (2013). Hybridization and speciation. Journal of Evolutionary Biology, 26(2), 229–246. https://doi.org/10.1111/j.1420-9101.2012.02599.x
Amo, L., Avilés, J. M., Parejo, D., Peña, A., Rodríguez, J., & Tomás, G. (2012). Sex recognition by odour and variation in the uropygial gland secretion in starlings. Journal of Animal Ecology, 81(3), 605–613. https://doi.org/10.1111/j.1365-2656.2011.01940.x
Amo, L., Galván, I., Tomás, G., & Sanz, J. J. (2008). Predator odour recognition and avoidance in a songbird. Functional Ecology, 22(2), 289–293. https://doi.org/10.1111/j.1365-2435.2007.01361.x
Amo, L., Visser, M. E., & van Oers, K. (2011). Smelling out predators is innate in birds. A rdea, 99(2), 177–184. https://doi.org/10.5253/078.099.0207
Apandi, M., & Edwards, H. M. (1964). Studies on the composition of the secretions of the uropygial gland of some avian species. Poultry Science, 43(6), 1445–1462. https://doi.org/10.3932/ps.0431445
Armitage, P., Berry, G., & Matthews, J. N. S. (1994). Statistical methods in medical research. Hoboken, NJ: John Wiley and Sons.
Balthazart, J., & Taziaux, M. (2010). The underestimated role of olfaction in avian reproduction? Behavioral Brain Research, 200(2), 248–259. https://doi.org/10.1016/j.bbr.2008.08.036
Bang, B. G., & Cobb, S. (1968). The size of the olfactory bulb in 108 species of birds. The Auk, 85, 55–61.
Barbosa, D., Font, E., Desfilis, E., & Carretero, M. A. (2006). Chemically mediated species recognition in closely related Podarcis wall lizards. Journal of Chemical Ecology, 32(7), 1587–1598. https://doi.org/10.1007/s10886-006-9072-5
Baum, C. F. (2008). Stat tip 63: Modeling proportions. The Stata Journal, 8(2), 299–303.
Bleidweiss, R. (2004). Ultraviolet plumage reflectance distinguishes sibling bird species. Proceedings of the National Academy of Sciences of the United States of America, 101(47), 16561–16564.
Bonadonna, F., & Sanz-Aguilar, A. (2012). Kin recognition and inbreeding avoidance in wild birds: The first evidence for individual kin-related odour recognition. Animal Behaviour, 84, 509–513. https://doi.org/10.1016/j.anbehav.2012.06.014
Bonadonna, F., & Mardon, J. (2010). One house two families: Petrel squatters get a sniff of low-cost breeding opportunities. Ethology, 116, 176–182. https://doi.org/10.1111/j.1439-0310.2009.01725.x
Bro-Jørgensen, J. (2009). Dynamics of multiple signaling systems: Animal communication in a world in flux. Trends in Ecology and Evolution, 25(5), 292–300.
Bronson, C. L., Grubb, T. C., Sattler, G. D., & Braun, M. J. (2003). Mate preference: A possible causal mechanism for a moving hybrid zone. Animal Behaviour, 65, 489–500. https://doi.org/10.1006/anbe.2003.2103
Bronson, C. L., Grubb, T. C., Sattler, G. D., & Braun, M. J. (2005). Reproductive success across the black-capped chickadee and Carolina chickadee hybrid zone in Ohio. The Auk, 122(3), 759–772.
Byers, B. E., & Kroodsma, D. E. (2009). Female mate choice and songbird song repertoires. Animal Behaviour, 77, 13–22. https://doi.org/10.1016/j.anbehav.2008.10.003
Byers, J. R., & Struble, D. L. (1990). Identification of sex-pheromones of two sibling species in dingy cutworm complex, Feltia jaculifera (GN.) (Lepidoptera: Noctuidae). Journal of Chemical Ecology, 16, 2981–2992. https://doi.org/10.1023/B:ECHE.00000797489
Campagna, S., Mardon, J., Celerier, A., & Bonadonna, F. (2011). Potential semiochemical molecules from birds: A practical and comprehensive compilation of the last 20 years studies. Chemical Senses, 37(1), 3–25. https://doi.org/10.1093/chemse/bjr067
Candolin, U. (2003). The use of multiple cues in mate choice. Biological Reviews, 78(4), 575–595. https://doi.org/10.1017/S146479310306158
Caro, S. P., & Balthazart, J. (2012). Pheromones in birds: Myth or reality? Journal of Comparative Physiology A, 196(10), 751–766. https://doi.org/10.1007/s00359-010-0534-4
Caro, S. P., Balthazart, J., & Bonadonna, F. (2015). The perfume of reproduction in birds: Chemosignaling in avian social life. Hormones and Behavior, 68, 25–42. https://doi.org/10.1016/j.ybeh.2014.06.001
Caspers, B. A., Gagliardo, A., & Krause, E. T. (2015). Impact of kin odour on reproduction in zebra finches. Behavioral Ecology and Sociobiology, 69, 1827-1833. https://doi.org/10.1007/s00265-015-1995-9

Caspers, B. A., Hagelin, J. C., Paul, M., Bock, S., Willeke, S., & Krause, E. T. (2017). Zebra finch chicks recognise parental scent, and retain chemosensory knowledge of their genetic mother, even after egg cross-fostering. Scientific Reports, 7, 12859.

Caspers, B. A., Hoffman, J. I., Kohlmeier, P., Krüger, O., & Krause, E. T. (2013). Olfactory imprinting as a mechanism for nest odour recognition in zebra finches. Animal Behaviour, 86, 85–90. https://doi.org/10.1016/j.anbehav.2013.04.015

Caubet, Y., Jaisson, P., & Lenoir, A. (1992). Preimaginal induction of adult behavior in insects. The Quarterly Journal of Experimental Psychology B, 44(3–4), 165–178.

Christophe, N., & Baudoin, C. (1998). Olfactory preferences in two strains of wild mice, Mus musculus musculus and Mus musculus domesticus, and their hybrids. Animal Behaviour, 56(2), 365–369. https://doi.org/10.1006/anbe.1998.0798

Clark, L., Avilova, K. V., & Bean, N. J. (1993). Odor thresholds in passerines. Comparative Biochemistry and Physiology A: Physiology, 104(2), 305–312. https://doi.org/10.1016/0300-9629(93)90322-U

Coffin, H. R., Watters, J. V., & Mateo, J. M. (2011). Odor-based recognition of familiar and related conspecifics: A first test conducted on captive Humboldt penguins (Spheniscus humboldti). PLoS ONE, 6(9), e25002. https://doi.org/10.1371/journal.pone.0025002

Courtenay, S. C. (1989). Learning and memory of chemosensory stimuli by underyearling coho salmon Oncorhynchus kisutch (Walbaum). Doctoral dissertation, University of British Columbia.

Coyne, J. A., Crittenden, A. P., & Mah, K. (1994). Genetics of a pheromone. Behavior Genetics, 24, 459–467. https://doi.org/10.1007/BF01041996

Delaney, E. K., & Hoekstra, H. R. (1992). Preimaginal induction of adult behavior in insects. Quarterly Journal of Experimental Psychology, 44(3–4), 165–178.

Demopoulos, W. B., Swanson, W. J., & Sorenson, M. D. (2005). Speciation in birds: Changes in male mate choice in a sexually cannibalistic orb-web spider (Araneae: Araneidae). Behaviour, 141(10), 1197–1210. https://doi.org/10.1163/1568539042729676

Dawley, E. M. (1984). Recognition of individual, sex and species odours by salamanders of the Plethodon glutinosus-P. jordani complex. Animal Behavior, 32(2), 353–361. https://doi.org/10.1016/S0003-3472(84)80268-7

De Leon, A., Mínguez, E., & Belliure, B. (2003). Self-odour recognition in European storm-petrel chicks. Behaviour, 140(7), 925–933.

Delaney, E. K., & Hoekstra, H. E. (2018). Diet-based assortative mating through sexual imprinting. bioRxiv, 338848. https://doi.org/10.1101/338848

Dawley, E. M. (1984). Recognition of individual, sex and species odours by salamanders of the Plemodon glutinosus-P. jordani complex. Animal Behavior, 32(2), 353–361. https://doi.org/10.1016/S0003-3472(84)80268-7

Dougherty, L. R., & Shuker, D. M. (2015). The effect of experimental design on the measurement of mate choice: A meta-analysis. Behavioral Ecology, 26(2), 311–319. https://doi.org/10.1093/beheco/aru125

Edward, D. A., & Chapman, T. (2011). The evolution and significance of male mate choice. Trends in Ecology and Evolution, 26(12), 647–654. https://doi.org/10.1016/j.tree.2011.07.012

Edwards, S. V., Kingan, S. B., Calkins, J. D., Balakrishnan, C. N., Jennings, W. B., Swanson, W. J., & Sorenson, M. D. (2005). Specification in birds: Genes, geography, and sexual selection. Proceedings of the National Academy of Sciences of the United States of America, 102, 6550–6557. https://doi.org/10.1073/pnas.0501846102

Fischer, I., Haliński, Ł. P., Meissner, W., Stepnowski, P., & Knitter, M. (2017). Seasonal changes in the prey wax composition of the hering gull Larus argentatus. Chemoecology, 27, 127–139. https://doi.org/10.1007/s00049-017-0239-z

Fox, J. (2007). “The car Package”. Vienna, Austria: R Foundation for Statistical Computing.

Gaskett, A. C., Herbertstein, M. E., Downes, B. J., & Elgar, M. A. (2004). Changes in male mate choice in a sexually cannibalistic orb-web spider (Araneae: Araneidae). Behaviour, 141(10), 1197–1210. https://doi.org/10.1163/1568539042729676
Servedio, M. R., & Noor, M. A. F. (2003). The role of reinforcement in speciation: Theory and data. Annual Review of Ecology, Evolution, and Systematics, 34, 339–364. https://doi.org/10.1146/annurev.ecols ys.34.011802.132412

Servedio, M. R., Van Doorn, G. S., Kopp, M., Frame, A. M., & Nosil, P. (2011). Magic traits in speciation: ‘magic’ but not rare? Trends in Ecology and Evolution, 26(8), 389–397. https://doi.org/10.1016/j.tree.2011.04.005

Shahandeh, M. P., Pischedda, A., & Turner, T. L. (2017). Male mate choice via cuticular hydrocarbon pheromones drives reproductive isolation between Drosophila species. Evolution, 72(1), 123–135.

Shaw, C. L., Rutter, J. E., Austin, A. L., Garvin, M. C., & Whelan, R. J. (2011). Volatile and semivolatile compounds in gray catbird uruphalial secretions vary with age and between breeding and wintering grounds. Journal of Chemical Ecology, 37(4), 329–339. https://doi.org/10.1007/s10886-011-9931-6

Singer, T. L. (1998). Roles of hydrocarbons in the recognition systems of insects. American Zoologist, 38, 394–405. https://doi.org/10.1093/icb/38.3.394

Slabbeekoom, H., & Smith, T. B. (2002). Bird song, ecology and speciation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 356, 493–503. https://doi.org/10.1098/rstb.2001.1056

Smadja, C., & Butlin, R. K. (2009). On the scent of speciation: The chemosensory system and its role in premating isolation. Heredity, 102, 77–97. https://doi.org/10.1038/hdy.2008.55

Sneddon, H., Hadden, R., & Hepper, P. G. (1998). Chemosensory learning in the chicken embryo. Physiology and Behavior, 64(2), 133–139. https://doi.org/10.1016/S0031-9384(98)00037-7

Snowberg, L. K., & Bolnick, D. I. (2008). Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. The American Naturalist, 172, 733–739. https://doi.org/10.1086/591692

Soini, H. A., Schrook, S. E., Bruce, K. E., Wiesler, D., Ketterson, E. D., & Novotny, M. V. (2007). Seasonal variation in volatile compound profiles of preen gland secretions of the dark-eyed junco (Junco Hyemalis). Journal of Chemical Ecology, 33(1), 183–198. https://doi.org/10.1007/s10886-006-9210-0

Soini, H. A., Whitaker, D. J., Wiesler, D., Ketterson, E. D., & Novotny, M. V. (2013). Chemosignaling diversity in songbirds: Chromatographic profiling of preen oil volatiles in different species. Journal of Chromatography A, 1317, 186–192. https://doi.org/10.1016/j.jchroma.2013.08.006

Sonnenberg, B. R., Branch, C. L., Pitera, A. M., Bridge, E., & Pravosudov, V. V. (2019). Natural selection and spatial cognition in wild food-caching mountain chickadees. Current Biology, 29(4), 670–676. https://doi.org/10.1016/j.cub.2019.01.006

Steiger, S. S., Fidler, A. E., Valcu, M., & Kempenaers, B. (2011). Vocalization and spatial orientation in the polymorphic white-throated sparrow. Journal of Chemical Ecology, 37(3), 535–542. https://doi.org/10.1007/s10886-006-0457-5

Taylor, S. A., White, T. A., Hochachka, W. M., Ferretti, V., Curry, R. L., & Lovette, I. (2014). Climate-mediated movement of an avian hybrid zone. Current Biology, 24(6), 671–676. https://doi.org/10.1016/j.cub.2014.01.069

Tuttle, E. M., Sebastian, P. J., Posto, A. L., Soini, H. A., Novotny, M. V., & Gonsor, R. A. (2014). Variation in preen oil composition pertaining to season, sex, and genotype in the polymorphic white-throated sparrow. Journal of Chemical Ecology, 40, 1025–1038. https://doi.org/10.1007/s10886-014-0493-2

Verrell, P. A. (1985). Male mate choice for large, fecund females in the red-spotted newt, Notophthalmus viridescens: How is size assessed? Herpetologica, 41(4), 382–386.

Verzijden, M. N., Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., & Svensson, E. I. (2012). The impact of learning on sexual selection and speciation. Trends in Ecology & Evolution, 27(9), 511–519. https://doi.org/10.1016/j.tree.2012.05.007

Wang, H. L., Zhao, C. H., & Wang, C. Z. (2005). Comparative study of sex pheromone composition and biosynthesis in Helicoverpa armigera, H. assulta and their hybrid. Insect Biochemistry and Molecular Biology, 35(6), 575–583. https://doi.org/10.1016/j.ibmb.2005.01.018

Warton, D. I., & Hui, F. K. C. (2011). The arcsine is asine: The analysis of proportions in ecology. Ecology, 92(1), 3–10. https://doi.org/10.1890/10-0340.1

Whelan, R. J., Levin, T. C., Owen, J. C., & Garvin, M. C. (2010). Short-chain carboxylic acids from gray catbird (Dumetella carolinensis) uruphalial secretions vary with testosterone levels and photoperiod. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 156(3), 183–188. https://doi.org/10.1016/j.cbpb.2010.03.005

Whittaker, D. J., & Gerlach, N. M. (2016). Mate choice in dark-eyed juncos using visual, acoustic, and chemical cues. In E. D. Ketterson, & J. W. Atwell (Eds.), Snowbird: Integrative biology and evolutionary diversity in the junco (pp. 281–309). Chicago, IL: University of Chicago Press.

Whittaker, D. J., Gerlach, N. M., Soini, H. A., Novotny, M. V., & Ketterson, E. D. (2013). Bird odour predicts reproductive success. Animal Behaviour, 86(4), 697–703. https://doi.org/10.1016/j.anbehav.2013.07.025

Whittaker, D. J., Kuzel, M., Burrell, M. J. E., Soini, H. A., Novotny, M. V., & DuVal, E. H. (2019). Chemical profiles reflect heterozygosity and seasonality in a tropical lekking passerine bird. Animal Behaviour, 151, 67–75. https://doi.org/10.1016/j.anbehav.2019.03.005

Whittaker, D. J., Richmond, K. M., Miller, A. K., Kiley, R., Burns, C. B., Atwell, J. W., & Ketterson, E. D. (2011). Intraspecific preen oil odor preferences in dark-eyed juncos (Junco Hyemalis). Behavioral Ecology, 22(6), 1256–1263. https://doi.org/10.1093/beheco/arq122

Whittaker, D. J., Rosvall, K. A., Slownski, S. P., Soini, H. A., Novotny, M. V., & Ketterson, E. D. (2018). Songbird chemical signals reflect uruphalial gland androgen sensitivity and predict aggression: Implications for the role of the periphery in chemosignaling. Journal of Comparative Physiology A, 204(1), 5–15. https://doi.org/10.1007/s00359-017-1221-5

Yang, Y., Blomenkamp, S., Dugas, M. B., Richards-Zawacki, C. L., & Pröhl, H. (2019). Mate choice versus mate preference: Inferences about color-assortative mating differ between field and lab assays of poison frog behavior. The American Naturalist, 193(4). https://doi.org/10.1086/702249
Yang, Y., Richards-Zawacki, C. L., Devar, A., & Dugas, M. B. (2016). Poison frog color morphs express assortative mate preferences in allopatry but not sympatry. *Evolution, 70*(12), 2778–2788. https://doi.org/10.1111/evo.13079

Zhang, J., Sun, L., & Zuo, M. (2009). Uropygial gland volatiles may code for olfactory information about sex, individual, and species in Bengalese finches *Lonchura striata*. *Current Zoology, 55*(5), 357–365.

Zhang, J., Wei, W., Zhang, J., & Yang, W. (2010). Uropygial gland-secreted alkanols contribute to olfactory sex signals in budgerigars. *Chemical Senses, 35*(5), 375–382. https://doi.org/10.1093/chemse/bjq025

Zhang, Y., Du, Y., & Zhang, J. (2013). Uropygial gland volatiles facilitate species recognition between two sympatric sibling bird species. *Behavioral Ecology, 24*(6), 1271-1278. https://doi.org/10.1093/beheco/art068

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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