



Odor preferences in hybrid chickadees: implications for reproductive isolation and asymmetric introgression

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Abstract

Preferences for mating cues can have important effects on speciation in natural hybrid zones. While mating preferences of parental species are well studied, hybrid preferences for cues of parental species have received less attention. Nevertheless, hybrid preferences have potentially important consequences for reproductive isolation and patterns of introgression. Here, we test preferences in wild-caught hybrid chickadees for odor cues from the parental species, black-capped and Carolina chickadees. Olfaction has been historically understudied in avian species, especially passerines, but much recent work has begun to show how olfactory cues play important roles in songbird ecology. In contrast to the strong conspecific odor preferences we previously found in pure-species chickadees, male hybrids as a group show no preference for the odor of either parental species, while still exhibiting individual instances of preference. Female hybrids show preferences for black-capped odor, but this signature is driven by individuals with elevated proportions of black-capped ancestry while Carolina-like female hybrids show no preferences. We discuss the implications of a lack of some female hybrid preferences on reproductive isolation, and the potential for asymmetric preferences in male and black-capped-like female hybrids to contribute to directional introgression and northward movement of the hybrid zone.

Significance statement

Mate preference has important implications for the fate of natural hybrid zones and the speciation process. Mating discrimination by pure species individuals against hybrids is commonly observed in hybrid zones. However, mate preferences of hybrid individuals for the parental species can be just as important. We previously found that hybrid zone black-capped and Carolina chickadees produce distinct odor profiles and show clear preferences for conspecific odor cues. Here, we assessed preferences of wild-caught hybrid chickadees for the odors of both parental species. In contrast to pure-species birds, hybrid males overall show no preference for either parental species odor as a group. Still, individual males do show clear preferences. Hybrid females show a preference for black-capped odor that is dependent on their own degree of black-capped ancestry. Carolina-like hybrid females show no preferences. Our results suggest the potential for hybrid preference to contribute to reproductive isolation and northward movement of the chickadee hybrid zone.

Keywords Speciation · Hybridization · Reproductive isolation · Olfaction · Black-capped chickadee · Carolina chickadee

Introduction

Understanding how mate choice behaviors maintain species boundaries is a central focus of speciation research. Natural hybridization — when distinct species mate and produce offspring — can provide important insights into how signals and mate preferences affect animal speciation (Jiggins and Mallet 2000; Panhuis et al. 2001). Postzygotic isolation in the form of sexual selection against hybrid individuals has been demonstrated in many animal taxa (Vamosi and Schluter 1999; Naisbit et al. 2001; Svedin et al. 2008;

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Lemmon and Lemmon 2010; Latour et al. 2013). However, hybrid preferences for parental species signals and whether they play a role in reproductive isolation and patterns of introgression have received less attention.

How hybrids perceive information and incorporate signals into their mate choice decisions is relatively unexplored. Fine-scale preference clines have been examined across hybrid zones (Butlin and Ritchie 1991; Ganem et al. 2008), but rarely do these studies specifically analyze the preferences of genetically admixed individuals. In addition, experiments demonstrating reduced mating probability for hybrids often do not differentiate between the potential reduced attractiveness of hybrids versus the possibility of compromised perceptions and preferences of hybrids (Davies et al. 1997; Naisbit et al. 2001; Muñoz et al. 2010).

Hybrid preferences can reveal important insights into mating dynamics within natural hybrid zones, affecting postzygotic isolation and influencing the amount and direction of introgression between parental species (Rosenthal 2013). For example, asymmetric preferences for parental species signals have been found in hybrid lacewings (*Chrysoperla plorabunda* and *C. johnsoni*) (Wells and Henry 1994), crickets (*Gryllus bimaculatus* and *G. campestris*) (Veen et al. 2013), and house mice (*Mus musculus musculus* and *M. musculus domesticus*) (Christophe and Baudoin 1998), and such biased preferences can influence patterns of introgression (Teeter et al. 2008). Additionally, female hybrid preferences in spadefoot toads (*Spea multiplicata* and *S. bombifrons*) change with environmental conditions, potentially affecting reproductive isolation and levels of asymmetric introgression (Schmidt and Pfennig 2016). In hybrid *Heliconius* butterflies, preferences for color patterns of other hybrids over parental species may lead to reproductive isolation between parental species as well as the formation of hybrid species (Melo et al. 2009). Here we examine the preferences of hybrid chickadees for the odor cues of their parental species, the black-capped (*Poecile atricapillus*) and Carolina chickadee (*P. carolinensis*).

The North American black-capped and Carolina chickadees are sister taxa (Harris et al. 2014) that hybridize along a narrow contact zone stretching from New Jersey to Kansas, USA (McQuillan and Rice 2015). This hybrid zone has been moving steadily northward due to climate change at the rate of approximately 10 km/decade (Taylor et al. 2014a, b; Wagner et al. 2020). Although successful interspecific matings occur, there seems to be strong selection against hybrids (Wagner et al. 2020). Postzygotic barriers are present in this system in the form of reduced hatching success for mixed-species parental pairs (Bronson et al. 2005; Van Huynh and Rice 2019a, b) and cognitive deficiencies in adult hybrids (McQuillan et al. 2018).

We previously found that sympatric black-capped and Carolina chickadees produce chemically distinct uropygial

gland oils, a major odor source in birds (Hagelin and Jones 2006), and that males and females of both species show clear preferences for conspecific odors over heterospecific odors (Van Huynh and Rice 2019a, b). Within the hybrid zone, odor may be an important mate choice cue because plumage and vocal signals can be unreliable in this system; both pure species possess similar plumage (Robbins et al. 1986), and individuals can learn heterospecific song (Kroodsmat et al. 1995). We found that these preferences were persistent throughout the year (Van Huynh and Rice 2019a, b). Indeed, year-round odor preferences could influence mate choice decisions. Chickadees overwinter in relatively stable flocks with an approximate 1:1 sex ratio (Desrochers 1988; Desrochers and Hannon 1989). During this time, individuals can often be found in close proximity to one another, and pair-bonds form prior to the actual breeding season, during initial flock formation in the fall or later into mid-winter (Odum 1941; Lemmon et al. 1997).

To investigate how hybrid preferences might contribute to reproductive isolation or influence introgression patterns, we tested preferences for parental species odors in wild-caught adult hybrid chickadees. As noted above, hybrid preferences are relatively unexplored generally, as are preferences for odor cues in songbirds. Therefore, we did not have a priori predictions on the magnitude or direction of odor preferences that might be present in hybrid chickadees.

Methods

Field methods and animal housing

We captured wild chickadees using mist nets at two geographically proximate sites within the hybrid zone in eastern Pennsylvania (Lehigh University 40° 36' 5.2" N, 75° 21' 34.1" W and Jacobsburg State Park 40° 47' 4.0" N, 75° 17' 34.7" W). Upon capture, we banded each bird and collected a small blood sample for ancestry (McQuillan et al. 2017) and sex determination (Griffiths et al. 1998). We captured 19 male and 12 female hybrids that were identified by genotyping (see genotyping methods below) between February 2017 and November 2018 and transported them to an outdoor aviary at Lehigh University. Live pure-species birds used in a separate odor preference project (Van Huynh and Rice 2019a, b) were used as odor-donor birds (11 black-capped males, 13 black-capped females, 9 Carolina males, 11 Carolina females). Odor-donor birds were used between 1 and 6 times, with the average bird being used in 1.4 trials. All birds were housed and maintained following Van Huynh and Rice (2019a, b). Because individuals were held for ~ 1 week for testing (see odor preference tests below), we did not test birds during the breeding season in order to avoid

disrupting breeding efforts (mid-March through July). However, chickadees' odor preferences can be relevant to mate choice decisions throughout the year. Pure-species preferences for conspecific odors were observed throughout the year (Van Huynh and Rice 2019a, b), and the formation of pair-bonds can occur in the fall or winter, months prior to the actual breeding season (Lemmon et al. 1997).

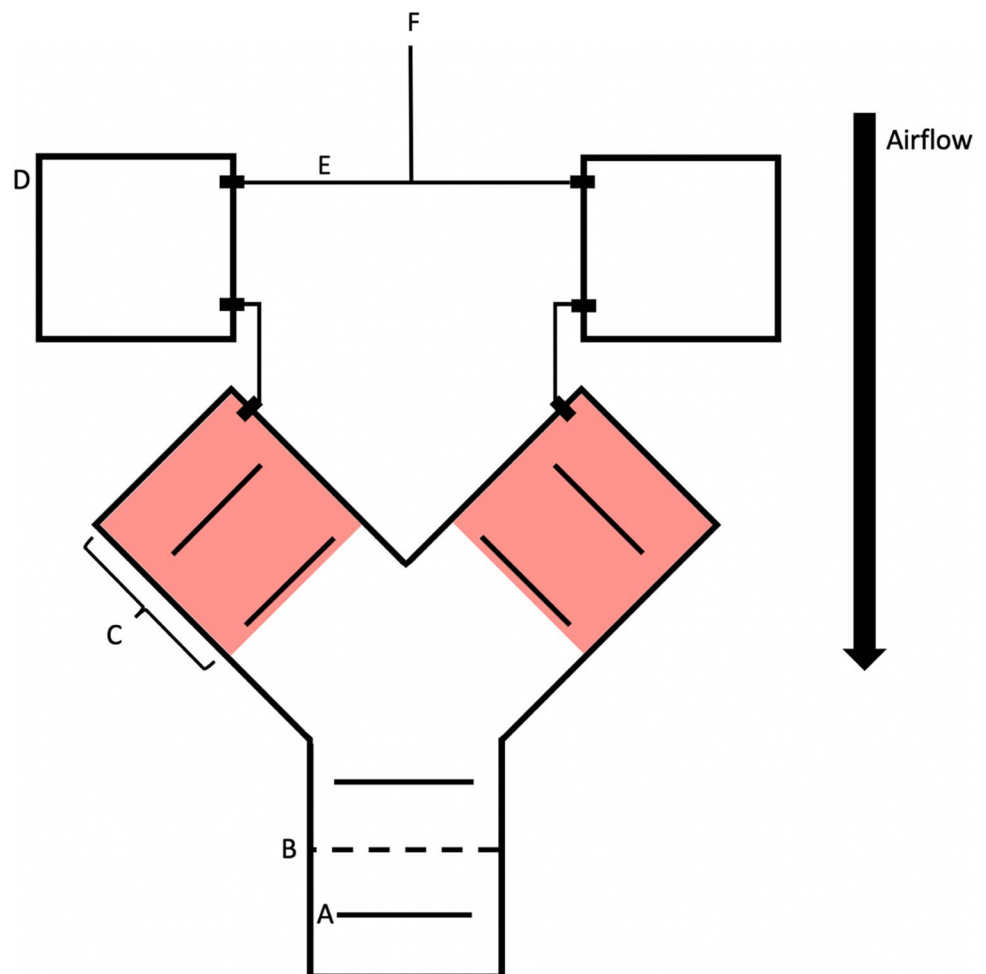
Genotyping

To assess the extent of hybrid ancestry, we first extracted genomic DNA from blood samples using a DNeasy blood and tissue kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. We then genotyped individuals using 10 species-diagnostic SNP markers (McQuillan et al. 2017) and the program STRUCTURE (Hubisz et al. 2009). We designated hybrids as individuals with admixture values falling outside the 90% credible interval of known pure individuals, following McQuillan et al. (2017) (see also McQuillan et al. 2018; Van Huynh and Rice 2019a, b).

Odor preference tests

Odor preference tests were carried out following the same methods as Van Huynh and Rice (2019a, b). Tests were carried out between February 2017 and November 2018. To test hybrid preferences for odors of parental species, we conducted sequential no-choice preference tests (Dougherty and Shuker 2015) using a Y-maze chamber (Fig. 1; height: 20 cm, length of choice arms: 45 cm, length of starting arm: 30 cm, width of choice arms and starting arm: 20 cm; also see Fig. 2 in Van Huynh and Rice (2019a, b)). Our Y-maze contained two wooden perches in each choice arm and in the starting arm (Fig. 1A). Three days after capture, each bird was acclimated to the chamber for 1 h with food supplied throughout the chamber to promote exploration. The first no-choice test with one parental species odor was conducted 3 days after acclimation, and the second no-choice test with the other parental species odor was conducted 3 days after the first test. During acclimation and during each no-choice test, air was pumped to both arms of the Y-maze so that air flow could be faintly felt at the ends of the arms. However, during the no-choice tests, one arm contained an odor

Fig. 1 Y-maze chamber for odor preference tests. Airflow direction is indicated on the right, with air moving into the odor-donor chambers and then into the Y-maze. (A) Wooden perch; (B) removable plexiglass divider; (C) choice area; (D) odor-donor chamber; (E) plastic tubing; (F) air input. Test birds were visually isolated from odor-donor birds. Odor-donor birds were maintained in the dark, and were therefore silent, during testing. Diagram is not drawn to scale (see main text for dimensions)



of either a live Carolina or black-capped chickadee of the opposite sex, which was randomly assigned to one of the two odor-donor chambers (20 cm × 20 cm × 20 cm; Fig. 1D). Pure black-capped and Carolina chickadees show significant interspecific differences in the chemical composition of their uropygial oils (Van Huynh and Rice 2019a, b), which are thought to be a major source of odor cues in birds (Hagelin and Jones 2006). Some odor-donor birds were used in multiple tests (20 Carolina and 24 black-capped individuals used in 62 total tests; see methods for statistical analyses below). The order of tests presented to each hybrid individual (black-capped versus Carolina odor donor) was determined randomly.

During each trial, the Y-maze and the odor-donor chambers were visually separated by an opaque divider. Two standing lamps illuminated the area of the room with the Y-maze, while odor-donor chambers in this study were confined to darkness so that the odor-donor birds remained still and silent. We have previously observed that chickadees are reliably nonactive in the dark (AVH, pers. obs.). Additionally, the ambient sound of airflow as it was pumped through the apparatus and the room masked any small amounts of noise or activity that we might not have noticed. In analyzing the video footage from the trials, we observed no vocalizations or other noises produced by the odor-donor birds.

At the beginning of each trial, the test bird was confined to the starting area (Fig. 1B) for 5 min before release into the Y-maze. Upon release, video recording began and the test bird was allowed to explore the Y-maze. The 15-min testing period began as soon as the test bird experienced both sides of the Y-maze (i.e., when the bird entered the arm of the Y-maze opposite its initial choice after release). The time spent by the bird in the odor arm of the Y-maze was analyzed from the videos. The bird was considered to be investigating the odor arm when it was on or beyond the wooden perch in the odor arm most proximal to the center of the Y-maze (Fig. 1C). All testing apparatuses were cleaned with 100% ethanol and allowed to air dry between trials. To minimize observer bias, video recordings were analyzed without knowledge of the identity of the focal bird or odor source.

Statistical analysis

We used paired two-tailed nonparametric Wilcoxon rank-sum tests to compare time spent by hybrid chickadees with black-capped versus Carolina 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 to total Y-maze volume × test duration (900 s) = 252 s) using one-sample Wilcoxon rank-sum tests.

Sampling distributions and estimations of effect sizes of differences in mean preference for Carolina versus

black-capped odor in male and female hybrids were calculated using bootstrapping by resampling with replacement (5000 resamples) using the R package *dabestr* and the corresponding webapp (www.estimationstatistics.com) (Ho 2019). Using the same package, we calculated 95% confidence intervals and plotted them against the null expectation of zero difference in mean preference. To compare hybrid preference with the preferences exhibited by parental chickadee species, we used the same approach to calculate sampling distributions and to estimate effect sizes for the preferences of pure-species individuals using data originally reported in Van Huynh and Rice (2019a, b; raw data deposited in the dryad repository: <https://doi.org/10.5061/dryad.7ht92p7>).

Birds designated as hybrids encompass a range of genetic admixture from F1 hybrids to advanced generation backcrosses in the direction of both parental species. Therefore, we also tested whether a continuous measure of ancestry explained variation in odor preference. We fit a linear mixed-effects model of the difference in time spent with black-capped versus Carolina chickadee odor by the fixed factors of sex and a continuous measure of ancestry using the R package *lme4* (Bates et al. 2015). Estimated proportions of black-capped ancestry were obtained from the STRUCTURE analysis (individual admixture values, *q*-values) described above. Since we previously found slightly weaker preferences for conspecific odors in female black-capped chickadees (Van Huynh and Rice 2019a, b), we also included a sex by ancestry interaction. We previously found no effect of date on odor preferences in pure-species birds (Van Huynh and Rice 2019a, b), so we excluded this factor from the analysis. We started with a full linear mixed-effects model, including the random effects of odor-donor IDs, to account for the repeated use of some odor-donor birds, and the order of the two tests (Wagner 1998). Because these random effects had near-zero variance estimates in the full model, resulting in a singular fit, the best-fit model was determined by a step-wise simplification procedure (Barr et al. 2013). We removed random effects one at a time and compared the inclusive and exclusive models using a likelihood ratio test. If the simplified model explained significantly less variation, this would suggest the random effect in question should be retained in our model. Using this model simplification procedure, all three random effects (black-capped odor donor ID, Carolina odor-donor ID, and test order) were removed (see [Supplementary Material](#)). The final simplified linear model, including only the fixed effects of sex, ancestry, and their interaction, was evaluated with a type-II ANOVA using the R package *car* (Fox and Weisberg 2019).

Lastly, we analyzed the strength of absolute preference in all hybrid individuals tested by taking the absolute value of the difference between the times spent in the odor arm over both trials. We fitted this value using a negative binomial

generalized linear model with the fixed factors of ancestry, sex, and their interaction using the R package *MASS* (Venables and Ripley 2002). This model was evaluated with a type-II ANOVA using the R package *car* (Fox and Weisberg 2019). All analyses and visualizations were conducted in R [4.0.2] (R Core Team 2020).

Results

Overall, male hybrids as a group showed no preference for one parental species odor over the other (Fig. 2A; $W=88$, $p=0.80$). Males also showed no preference for the odor of either parental species compared to random movement

(black-capped odor $W=89$, $p=0.83$; Carolina odor $W=73$, $p=0.40$). This lack of preference in males as a group was similarly reflected in the non-significant effect size of the mean difference in time spent with Carolina chickadee odor minus the time spent with black-capped chickadee odor estimated by bootstrapping (Fig. 2B; mean = 41.1, 95% CI = [-66, 173]). On the other hand, when taken together, our data suggest a possible weak preference in female hybrids for black-capped over Carolina odor. The Wilcoxon rank-sum tests showed a suggestive yet non-significant preference in female hybrids for black-capped odor over Carolina odor (Fig. 2A; $W=14$, $p=0.052$). Compared to random movement, female hybrids showed no significant preference for Carolina odor ($W=36$, $p=0.85$) but seemed to trend

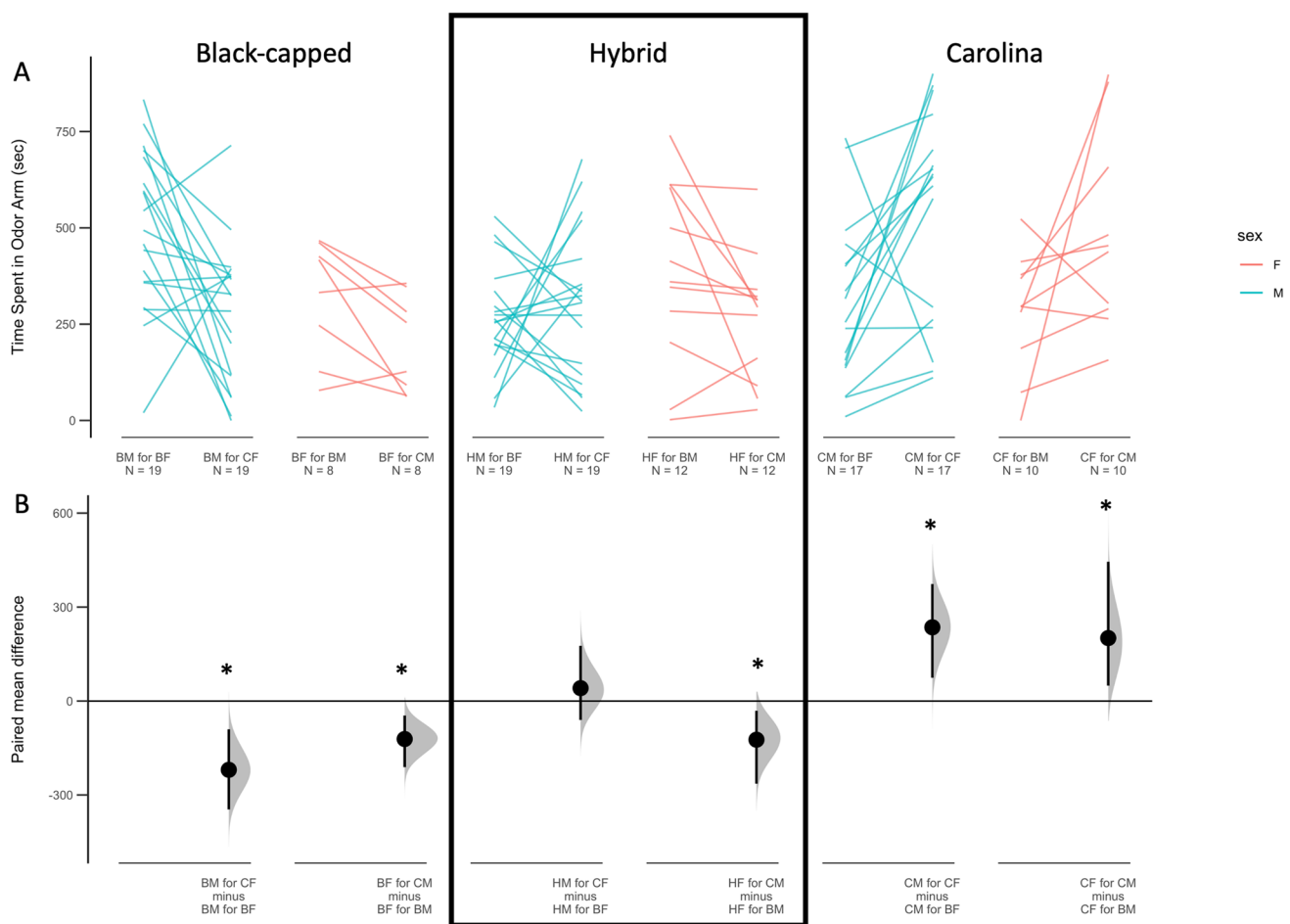


Fig. 2 (A) Odor preferences of hybrid chickadees (middle panel) for black-capped (BM males, BF females) versus Carolina (CM, CF) odor. For comparison, data from Van Huynh and Rice (2019a, b) showing significant conspecific preferences in pure black-capped (left panel) and Carolina chickadees (right panel) were reanalyzed and are also illustrated here. Captions denote the time spent in the odor arm during specific 15-min trials (e.g., HM for BF; hybrid males for

black-capped female odor). (B) Bootstrapped sampling distributions and effect sizes for the time difference within each group are plotted against the null expectation of zero with 95% confidence intervals. Asterisks indicate a significant deviation from zero in the bootstrapped sampling distribution (see results from Van Huynh and Rice 2019a, b)

Table 1 Analysis of variance (type-II tests) of the difference between the time spent by hybrid birds between black-capped and Carolina odors. Results are from a linear model with the fixed factors of ancestry, sex, and their interaction. Significant effects are highlighted in bold and indicated with an asterisk

	<i>F</i>	<i>df</i>	<i>P</i>
Ancestry	1.883	1	0.1813
Sex	4.607	1	0.0410*
Ancestry × sex	4.208	1	0.0501

toward preferring black-capped odor ($W = 16$, $p = 0.077$). Finally, the bootstrapped sampling distribution suggests that the estimated effect size of black-capped preferences in female hybrids was significant (Fig. 2B; mean = -124 , 95% CI = $[-262, -34]$). Bootstrapped effect sizes for pure species from Van Huynh and Rice (2019a, b) were calculated as well for comparison (Fig. 2B). Black-capped males (mean = -220 , 95% CI = $[-349, -85]$) and females (mean = -121 , 95% CI = $[-212, -46]$) and Carolina males

(mean = 235 , 95% CI = $[64, 368]$) and females (mean = 201 , 95% CI = $[47, 446]$) showed significant effect sizes for conspecific odor preference.

We also used a linear model to examine the effects of sex and a continuous measure of ancestry on preference for black-capped over Carolina odors. We found a significant effect of sex (Table 1, Fig. 3, $F(1) = 4.607$, $p < 0.05$) and a near-significant interaction effect of sex by ancestry (Table 1, Fig. 3, $F(1) = 4.208$, $p = 0.0501$). Specifically, these results appeared to be driven by three female individuals possessing high levels of black-capped ancestry exhibiting strong preferences for black-capped odor over Carolina odor (Fig. 3).

Because it appeared that hybrid preferences were present at the individual level (Fig. 2A), we used a negative binomial generalized linear model to examine the strength of absolute preference in hybrids (i.e., whether individuals preferred any one odor over another) by taking the absolute value of the difference in time spent in the odor arms over the two trials. We found a significant interaction effect of

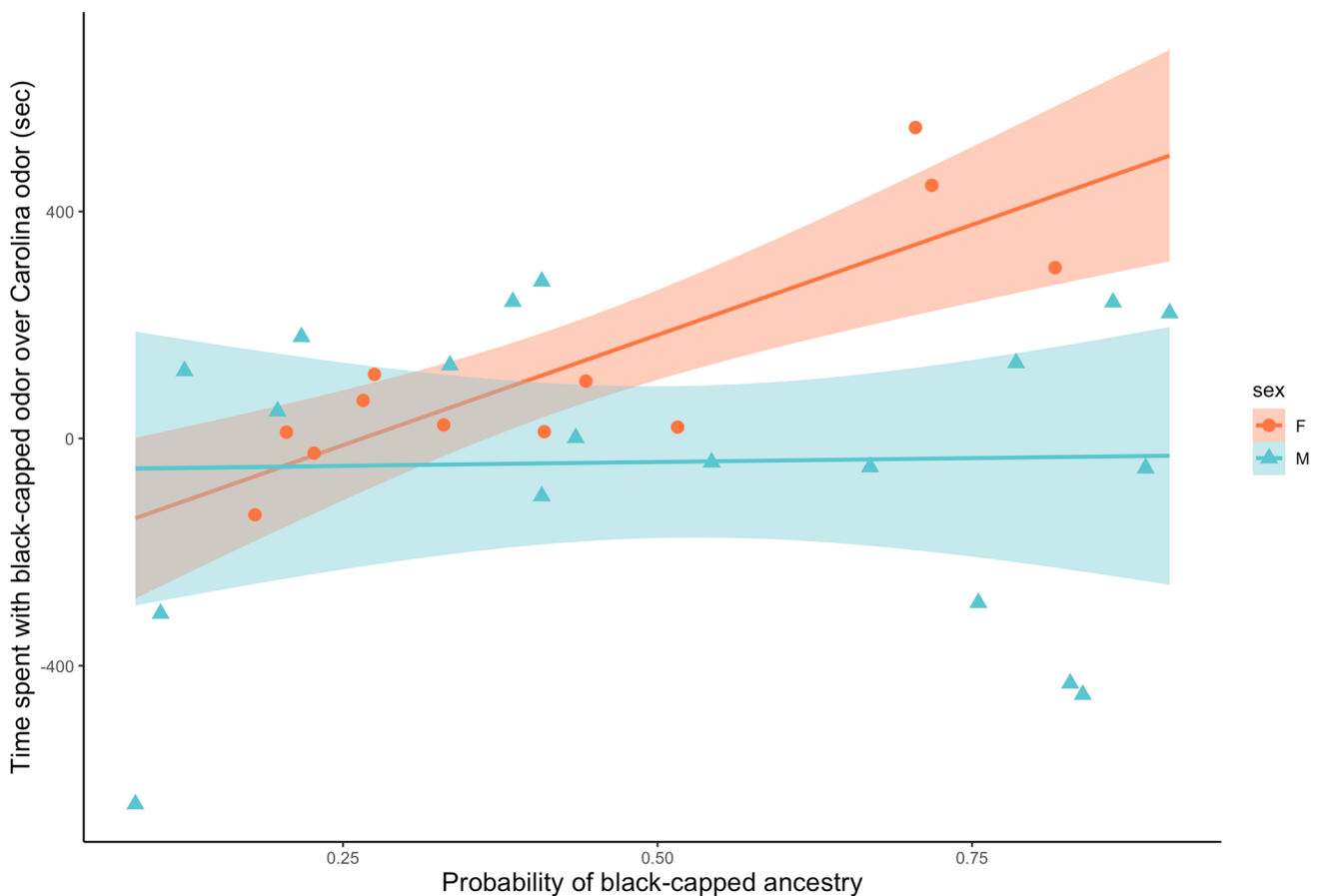


Fig. 3 Time spent with black-capped odor over Carolina odor by male and female hybrid chickadees. The *x*-axis designates a continuous measure of ancestry with 0 corresponding to pure Carolina and 1

corresponding to pure black-capped ancestry. Linear regression lines with 95% CI are for males (blue) and females (red)

Table 2 Analysis of variance (type-II tests) of the absolute difference in time spent by hybrid birds between black-capped and Carolina odors. Results are from a negative binomial generalized linear model. Significant effects are highlighted in bold and indicated with an asterisk

	χ^2	df	P
Ancestry	2.139	1	0.1436
Sex	1.514	1	0.2185
Ancestry $\times$ sex	7.588	1	0.0059*

sex by ancestry (Table 2, Fig. 4, $\chi^2(1)=7.588$, $p < 0.01$). Interestingly, while male hybrids did not show any consistent directional preferences as a group, they did seem to show absolute preferences at the individual level independent of their proportion of admixture, indicated by the non-zero intercept of the male regression line (Fig. 4). In contrast, female hybrids did exhibit increasing strength of absolute preference, where black-capped-like females showed a clear instance of preference while females that were Carolina-like or of intermediate ancestry seemed to show no preference at all (Table 2, Fig. 4).

Discussion

Hybrid preferences for cues of parental species can have important implications for hybrid zone dynamics and speciation. Odor preferences (or lack thereof) in chickadees may influence mate choice decisions directly. Because pair-bonds can be formed throughout the year, even many months prior to the breeding season (Lemmon et al. 1997), odor preferences in chickadees may also indirectly affect mate choice through general social associations within winter flocks. We previously found that sympatric black-capped and Carolina chickadees exhibit strong preferences for conspecific odor throughout the year (Fig. 2; Van Huynh and Rice 2019a, b). In contrast, in the same population, we report a lack of consistent preferences in adult hybrid male chickadees as a group for black-capped or Carolina chickadee odor cues (Fig. 2). At the individual level, hybrid males did show a significant absolute preference for one odor over another (Figs. 2A and 4). However, the specific direction of these preferences was not explained by the proportion of parental species ancestry (Fig. 3). It is currently unknown what variables determine the direction of this preference. Other factors linked to mate choice in chickadees, such as rank (Otter et al. 1999), mate protection (Lemmon et al. 1997), and dominance (Bronson et al. 2005; Grava et al. 2012), may be signaled by odor cues (Whittaker et al. 2018). Further, there is evidence that odor can predict overall reproductive success in other bird species (Whittaker et al. 2013). If hybrid males with certain preferences are more likely to be successful in

mating or more fertile, the possibility could exist for directional introgression to be mediated by hybrid male choice.

Female hybrids showed a significant preference for black-capped male versus Carolina male odors (Fig. 2). When using a continuous measure of ancestry, hybrid females show a stronger preference for black-capped odor over Carolina odor than do hybrid males (Table 1, Fig. 3) and effect seems to be driven by three females with high proportions of black-capped ancestry (Table 1, Fig. 3). While our sample size of hybrid females with greater proportions of black-capped ancestry is relatively small, the bootstrapping analysis (Fig. 2B) indicates that their preferences are more extreme than expected by chance under a null hypothesis of no difference. These results indicate asymmetries in preference among hybrid individuals based on sex and levels of admixture. If preferences of black-capped-like hybrid females for black-capped odor translates to mate choice decisions in the wild, asymmetrical gene flow of Carolina alleles into black-capped populations could result. Although only low levels of overall introgression have been observed in the chickadee hybrid zone, this possibility is consistent with the observed northward movement of the hybrid zone (Harris et al. 2014; Taylor et al. 2014a, b; Wagner et al. 2020) and a subset of Carolina alleles showing patterns of northward introgression (Taylor et al. 2014b). Similar asymmetric preferences in the house mouse hybrid zone match observed patterns of directional genetic introgression (Vanlerberghe et al. 1986; Christophe and Baudoin 1998; Teeter et al. 2008). Additional testing of female hybrids with high levels of black-capped ancestry is necessary to evaluate the consistency of this directional preference, and in turn, the potential for asymmetric gene flow.

Interestingly, hybrid females that were more Carolina-like not only seemed to show no preference as a group (Table 1, Fig. 3), but they showed no absolute preference at the individual level either (Table 2, Fig. 4). A lack of such preferences could contribute to post-zygotic isolation between the black-capped chickadee and Carolina chickadee. If these hybrid females are unable to take advantage of a potential mate choice cue to the same extent as parental species individuals, they may be less successful in finding a mate and reproducing. Along with the other postzygotic barriers acting in chickadees, including reduced hatching success of mixed-species pairs (Bronson et al. 2005; Van Huynh and Rice 2019a, b) and reduced cognitive abilities of hybrids (McQuillan et al. 2018), a lack of odor preferences in female hybrids is consistent with the observed narrowness and stability of the hybrid zone (Bronson et al. 2003; Taylor et al. 2014a). Although the results presented here do not directly support this idea, they suggest an interesting avenue of future study. Behavioral experiments addressing this possibility should aim to measure actual instances of olfactory-mediated mate choice in female hybrids in the wild.

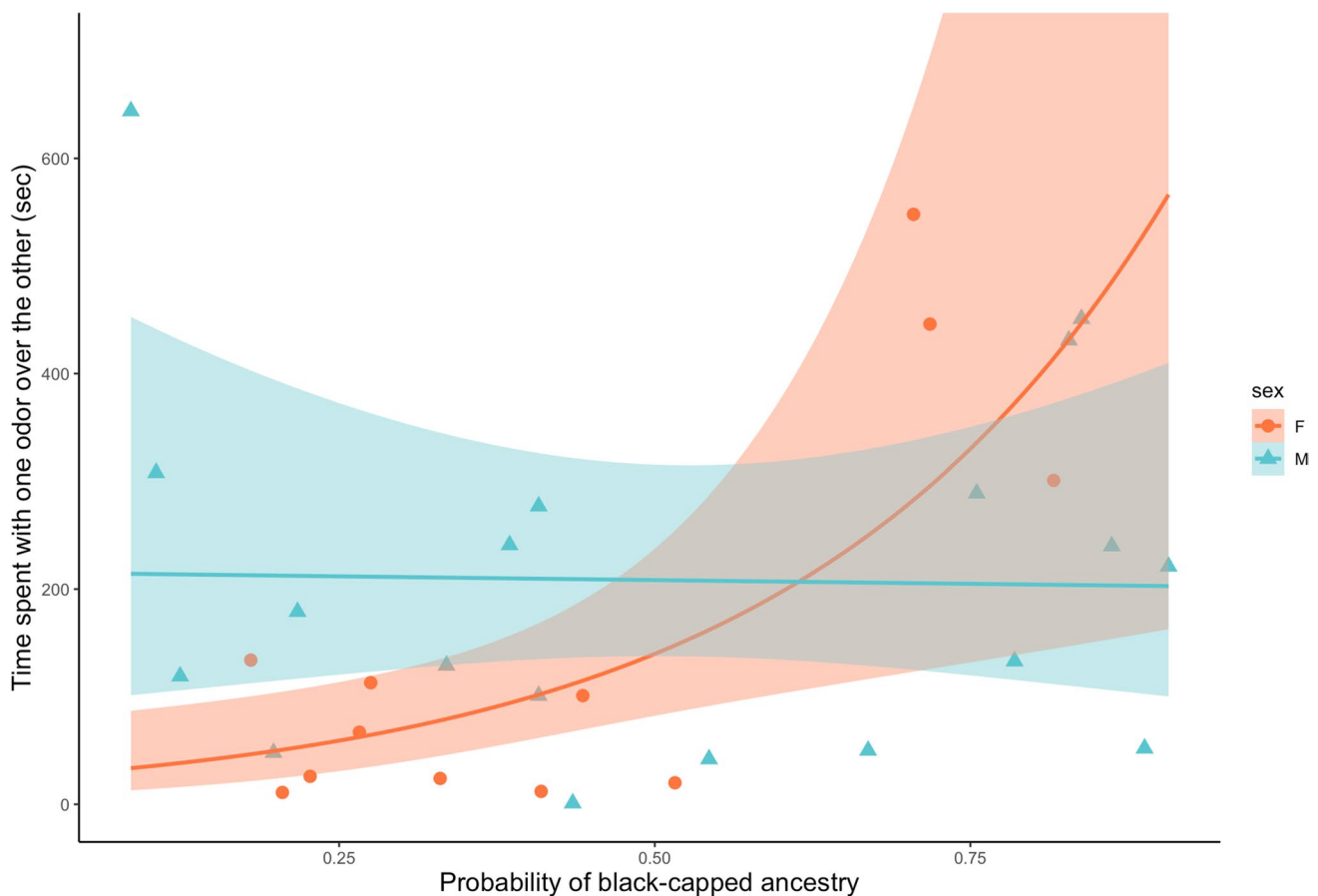


Fig. 4 Time spent with one odor over the other by male and female hybrid chickadees (i.e., absolute preference). The x-axis designates a continuous measure of ancestry with 0 corresponding to pure Carolina and 1 corresponding to pure black-capped ancestry. Negative

binomial fits with 95% CI are for males (blue) and females (red). Note that the regression line intercept for male hybrids is non-zero (see “Results”), illustrating the presence of significant preferences among individual males

The mechanism behind the absence of preferences in Carolina-like hybrid females for odors of parental species remains unknown. Since females are the heterogametic sex in birds, hybrid females are more likely to suffer fitness defects than hybrid males (Haldane 1922; Delph and Demuth 2016). One possibility is these hybrids have a reduced ability to detect odor cues, a phenomenon observed in hybrid *Rhagoletis* flies (Linn et al. 2004; Olsson 2006). If this is the case, hybrid females with reduced olfactory abilities may experience additional fitness consequences. It is unknown to what extent olfactory cues are incorporated into other behaviors in chickadees, but some songbird species use odor cues in recovering food caches (Buitron and Nuechterlein 1985) and assessing predation risk (Amo et al. 2008; Roth et al. 2008; Stanbury and Briskie 2015). Another possibility is that the cognitive processes involved in translating these cues into mate choice behaviors are dysfunctional. A recent genomic study found that some loci specifically associated with neuronal signaling are resistant to introgression in a geographically proximate transect of

the chickadee hybrid zone, underlying strong reproductive isolation and selection against hybrids (Wagner et al. 2020). Previous behavioral work has shown that hybrid chickadees are deficient in spatial memory and problem-solving tasks compared to pure-species birds, and that this deficiency was worse in hybrid females (McQuillan et al. 2018). While little else is known about the exact neurological bases and extent of these cognitive deficiencies, our results raise the possibility that additional neuronal processes, such as those involved in assimilating information through odor cues, may be compromised in hybrids. Also, whether odor preferences in pure-species chickadees are learned or innate is unknown, but nestling and embryonic imprinting on odor cues has been found in other avian species (Cunningham et al. 2003; De Leon et al. 2003; Caspers et al. 2017). Similar neuronal deficiencies that contribute to reduced learning and memory abilities in hybrid chickadees may also play a role in preventing hybrids from correctly imprinting on parental species odor cues. Future work on the chemical ecology of chickadees as well as the mechanisms of cognitive deficits

in hybrid chickadees may provide further insights into the findings presented here.

In many avian systems, chemical signaling via uropygial gland oils seems to be emphasized more in female individuals, which may facilitate intersexual advertisement of receptivity to male individuals (Whittaker and Hagelin 2021). Our results here are consistent with this idea in that hybrid males showed a greater instance of preference for pure-species odor cues (Table 2, Fig. 4) than did Carolina-like females. Additionally, while we previously observed clear conspecific odor preferences in pure-species males, pure black-capped females seemed to show slightly weaker preferences (Van Huynh and Rice 2019a, b). Conversely, directional odor preferences in a small number of black-capped-like female hybrids in our study may point to the possibility that female songbirds generally may exhibit emphasized reliance on odor signals compared to males. Because female birds in general usually exhibit larger uropygial glands during breeding and produce more complex and dynamic odor profiles than males (Hagelin and Jones 2006; Whittaker and Hagelin 2021), it could be that females are likewise more sensitive to odor cues as well. Although an interesting possibility, our results are conflicting on this. Our findings show that female hybrids overall show a greater preference for black-capped odor than male hybrids as a group (Table 1, Figs. 2 and 3); however, this result seems to be driven in large part by those females with high levels of black-capped ancestry, while those with more Carolina-like ancestry seem to show no preference at all (Table 2, Fig. 4). We suggest that more work needs to be done in songbirds generally to determine if the use of odor cues in determining behavior, both in the context of mate choice and in other ecologically relevant contexts such as foraging (Buitron and Nuechterlein 1985) or predator avoidance (Amo et al. 2008; Roth et al. 2008; Stanbury and Briskie 2015), shows sex-based patterns.

The lack of preferences for parental species odor cues in Carolina-like hybrid females may contribute to reproductive isolation between the black-capped and Carolina chickadee. Additionally, asymmetric olfactory preferences for black-capped odor cues in female hybrids with elevated levels of black-capped ancestry may contribute to low levels of northward introgression and accelerate the climate-mediated northward movement of the hybrid zone (Taylor et al. 2014a, b). While males as a group exhibited no significant preferences, individuals seemed to show a preference for one parental species odor over the other. This opens up the possibility for odor preferences to contribute to directional introgression depending on the as-yet-unknown factors that determine these preferences. The results presented here, along with future investigations of hybrid preferences in other animal hybrid zones, especially those with well documented genomic signatures of reproductive isolation or

introgression, will help us to better understand the role that hybrid preferences play in speciation.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-021-03069-2>.

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Author contribution AVH conceived of the project idea, performed experiments, and analyzed data. AVH and AMR designed experiments and wrote the manuscript.

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Data availability All pertaining data files and R code have been provided as [supplemental material](#).

Code availability All pertaining data files and R code have been provided as [supplemental material](#).

Declarations

Ethics approval Animal capture, collection, and transport were conducted under the U.S. Geological Survey federal bird banding permit 23810, U.S. Fish and Wildlife Service permit MB69567A-0, Pennsylvania Game Commission permits 103 and 145, and Pennsylvania Bureau of State Parks permit 2016–18. All applicable international and national guidelines for the use of animals were followed and all housing and testing procedures were approved by Lehigh University's Institutional Animal Care and Use Committee (Protocol #215).

Consent to participate Both authors have agreed to the participation of this submission.

Consent for publication Both authors have agreed to the publication of this submission.

Conflict of interest The authors declare no competing interests.

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