Do phylogeny and habitat influence admixture among four North American chickadee (family: Paridae) species?

Abstract:

Hybridization is an important aspect of speciation, yet questions remain about the ecological and environmental factors that influence hybridization among wild populations. We used microsatellite genotyping data and collected land cover and environmental data for four North American chickadee species: Black-capped (*Poecile atricapillus*), Mountain (*P. gambeli*), Chestnut-backed (*P. rufescens*), and Boreal (*P. hudsonicus*) Chickadees. Combining these datasets, we sought to examine whether there is evidence of admixture between four widely distributed North American chickadee species; whether admixture takes place more often between more closely related species pairs or between species pairs with more similar ecological preferences; and whether certain habitat types have higher rates of admixture than others. We detected admixture for five of the six species pairs analyzed (Chestnut-backed-Mountain Chickadee pair showed no evidence of admixture), and found rates of admixture varied geographically, and within taxa pairs. Admixture was higher among less closely related species than more closely related species, although habitat similarity was not a significant predictor. Finally, rates of admixture were higher in urban parkland habitats than deciduous, mixed, or coniferous forest habitats. Our work indicates admixture occurs frequently among North American Parids, and habitat and environmental variation may play an important role in the frequency and geographic distribution of hybridization.

Key words: *admixture, chickadee, habitat, hybridization, Paridae*

Introduction:

Hybridization plays an important role in speciation and the generation of species diversity (Soltis and Soltis 2009), but can also lead to genetic homogenization and the collapse of species pairs (Taylor et al. 2006, Seehausen et al. 2008, Behm et al. 2010). Genetic studies of species pairs in the wild offer the ideal systems to examine rates of admixture and hybridization in the context of ecological variation, including habitat, diet, dispersal, and social behaviour (Randler 2006). Testing these questions is especially important in the context of climate change and anthropogenic mediated habitat changes, to provide insights into the factors promoting hybridization in wild populations (Taylor et al. 2015, Billerman et al. 2016, Grabenstein and Taylor 2018).

Rates of admixture and hybridization are influenced through a variety of factors including prezygotic and postzygotic reproductive isolation (Borge et al. 2005, Lemmon and Lemmon 2010, Moran et al. 2019); for example, reductions in hybrid fitness appear to have a greater effect on reproductive isolation than assortative mating (Irwin 2020). Importantly, rates of admixture and hybridization can vary substantially across geographic space (Bell 1996, Lemmon and Juenger 2017), and therefore studies that examine admixture and hybridization across geographic space can provide greater insights into the processes facilitating admixture and hybridization between species pairs.

Hybridization is common in natural populations. Although it was previously thought that hybridization occurred exclusively between closely related species (Dasmahapatra et al. 2007), it is now recognized that hybridization occurs between deeply divergent lineages (Jasso-Martínez et al. 2018, Joseph et al. 2019, Tea et al. 2020). Questions remain about the processes that facilitate the events; for example does hybridization occur because species live in sympatry or does hybridization occur because species exhibit ecological or habitat similarities (Willis et al. 2014, Wood et al. 2016, Kyogoku and Kokko 2019)? Examining patterns of hybridization within areas of sympatry will assist in determining the interplay between hybridization and ecological factors.

Hybridization has been documented within the family Paridae in both North America and Europe (see Curry 2005). Among North American Parids, hybridization is well established for Carolina (*Poecile carolinensis*) and Black-capped Chickadees (*P. atricapillus*; Reudink et al. 2006, 2007, Taylor et al. 2014, Wagner et al. 2020), Tufted (*Baeolophus bicolor*) and Black-crested Titmouse (*B. atricristatus*; Curry and Patten 2014), and less frequently Black-capped and Boreal Chickadees (*P. hudsonicus*; Lait et al. 2012), and Mountain (*P. gambeli*) and Black-capped Chickadees (Grava et al. 2012). Recent analysis of phenotypic data suggests that hybridization occurs in 0.19% of all wild parids and that among passerines, Paridae hybridize most frequently (Justyn et al. 2020). Given how widely distributed parids are in North America, and the frequency of hybridization within this family, species within family Paridae are ideal for examining patterns of admixture in the context of ecological and habitat variation (Curry 2005). In this study we used five diagnostic microsatellite markers to examine patterns of admixture among four North American Chickadee species: Black-capped, Mountain, Boreal, and Chestnut-backed Chickadees across their ranges.

Here we test the following questions: 1) Do we see evidence of admixture between four widely distributed North American chickadee species? 2) Does admixture occur more frequently between more closely related species pairs (i.e. between species within the black-headed clade or brown-headed clade) or between species pairs with more similar ecological preferences? And 3) Do certain habitat types have higher rates of admixture than others?

Methods:

We examined patterns of admixture between four chickadee species in North America: Black-capped, Mountain, Chestnut-backed, and Boreal Chickadees. All four species are year round residents with varying degrees of overlap across their distribution range. The Black-capped Chickadee is the most abundant and widely distributed of the four species (Figure 1), and is often considered a generalist based on habitat characteristics; Black-capped Chickadees are commonly found in deciduous forests, mixed deciduous and coniferous forests, and open parkland areas (Table 1). The remaining three species are more habitat specialists and primarily restricted to specific habitat types. Both the Mountain and Chestnut-backed Chickadees have much smaller distributions compared to Black-capped and Boreal Chickadees and are exclusively found in western North America. Both species primarily occur in coniferous forests, although the Mountain Chickadee is more common in drier habitats, such as montane coniferous forests, and at higher elevations whereas the Chestnut-backed Chickadee occupies wetter and more densely vegetated coniferous forests and lower elevations. Similar to the Black-capped Chickadee, the Boreal Chickadee has a broad distribution across northern North America, and is commonly found in spruce forests. These species also offer interesting comparisons because of their phylogenetic history. Black-capped and Mountain Chickadees are both part of the black-headed clade, whereas Boreal and Chestnut-backed Chickadees are part of the brown-headed clade (Harris et al. 2014). We downloaded publicly available sequences (n=34) from the Barcode of Life Data system (http://www.boldsystems.org/) and analyzed a 652 bp sequence of Cytochrome Oxidase I gene to calculate divergence between the four species. We calculated mean group genetic distance between the four species in Mega X (Stecher et al. 2020) using a Jukes-Cantor model. Black-capped and Mountain Chickadees exhibited 5% sequence divergence, whereas divergence between Boreal and Chestnut-backed Chickadees is 3%. All comparisons between brown-headed and black-headed clades exceeded 5.8% (range=5.8%-7.2%).

A previous study by Grava et al. (2012) detailed the extent of hybridization between Mountain and Black-capped Chickadees within a hybrid zone in northern British Columbia, and we used this study as a guideline to select sites to examine admixture among North American Chickadee species. We identified areas of range overlap using QGIS 3.10 (QGIS.org 2021, Geographic Information Software, QGIS Association, http://www.qgiis.org). We obtained digital range maps from Bird Life

International (2020) and used the interpolation geoprocessing tool in QGIS to identify areas of overlap between species pairs (Figure 2). We then used the field calculator to calculate the amount of area overlap between species (Table 2). Following this step, we selected individuals from areas where species ranges overlap, and from areas where we captured two or more species from the same or nearby sites (e.g. southern Alberta, and central Alberta and Saskatchewan). Overall we analyzed genetic data from 780 Black-capped Chickadees, 280 Boreal Chickadees, 168 Chestnut-backed Chickadees, and 200 Mountain Chickadees. We chose three allopatric populations (one for Black-capped, Chestnut-backed, and Mountain Chickadees, locations shown in Figure 2) and used Labrador for Boreal Chickadees (as we had no other populations that qualified as allopatric for this species) to examine how genetically distinct species are in allopatry. For this comparison, we used a Principal Coordinate Analysis in GenAlEx 6.5 (Peakall and Smouse 2012) to examine how genetically distinct species are in allopatry (scatter plots are shown in Figure S1).

We used genotyping data from existing datasets (Burg et al. 2006, Grava et al. 2012, Lait and Burg 2013, Adams and Burg 2014, 2015, Adams et al. 2016, Hindley et al. 2018). The four species were genotyped at five diagnostic microsatellites: Escu6 (Hanotte et al. 1994); Titgata2, Titgata39 (Wang et al. 2005); Pdo5 (Griffith et al. 1999); and Pat14 (Otter et al. 1998); these five loci showed high resolution in being able to distinguish between the four species. All PCR conditions followed those outlined in Burg et al. (2006), Grava et al. (2012); Lait and Burg (2013), Adams and Burg (2014), and Hindley et al. (2018). We added genotyping data for Black-capped Chickadees at one microsatellite locus (Pdo5; n=780) and for Chestnut-backed Chickadees at two microsatellite loci (Titgata2 and Titgata39; n=168). A subset of samples from each species (n=20 per species) was run together on a 6% acrylamide gel with a Licor 4300 DNA analyzer (Licor Inc., Lincoln, NE) to calibrate the genotyping data from the multiple datasets.

Genetic admixture analyses

From the existing datasets, we analyzed nineteen different chickadee species pairs from 13 geographic regions (Figure 2; Table 3). Although as many as four chickadee species occur in sympatry in some areas, we restricted our analyses to chickadee pairs only, and therefore we analyzed multiple species pairs from some sites. For each geographic area, we used genotyping data from one to five populations for each species. We only combined populations from the same geographic area if previous studies had revealed them to be from the same genetic cluster (Burg et al. 2006, Lait and Burg, 2013, Adams and Burg 2014, Hindley et al. 2018) and the sites contained similar land cover. Individuals were assigned to a species based on their phenotypic appearance. Only one individual had a hybrid phenotype (Lait et al. 2012); this individual was a Boreal and Black-capped Chickadee hybrid. We used STRUCTURE v 2.3.3 (Pritchard et al. 2000) to examine admixture for each species pair and analyzed one to seven datasets for each species pair. For each STRUCTURE run, we used the correlated allele frequencies and admixture models with no loc priors. We analyzed K=2 for each species pair, for five separate runs, with a burn in of 50 000 and a post-burn in of 100 000 MCMC chain steps.

Following our STRUCTURE runs, we calculated the proportion of admixed individuals for each species at each site. For the purposes of this study, we considered an individual as admixed if the Q-value from our STRUCTURE analysis was >0.5 for the other species. We used a Kruskal-Wallis test to compare rates of admixture among pairs of chickadee species; the proportion of individuals showing admixture for each species from each site was used as the dependent variable and species as our independent variable. We compared patterns of admixture between sister species (Black-capped and Mountain Chickadees, and Boreal and Chestnut-backed Chickadees) and all non-sister species comparisons with a Kruskal-Wallis test. For these analyses, we used the combined proportion of admixed individuals between species pairs as our dependent variable and two categories, sister species and non-sister species, as our independent variable. Both analyses were run in PAST 3.0 (Hammer et al. 2001).

To determine the direction of gene flow between species pairs, we compared the frequency of admixed individuals within each species pair. For example, do admixed individuals more frequently have Black-capped Chickadee phenotypes in areas of contact with Mountain Chickadees? To examine these patterns we used Chi-Squared tests in R 3.6.3 (R Core Team, 2020) and compared the frequencies within the five species pairs where admixture was detected.

Habitat Variation

To compare habitat between the four species, we used four variables: land cover, elevation, mean annual temperature, and mean annual precipitation from each unique sampling point (i.e. bird's location). We collected land cover data from the North

American land change monitoring system from each site (Commission for Environmental Cooperation, 2010) which includes 19 different habitat classifications and we categorized land cover into four habitat categories: urban parkland, deciduous forest, mixed forest, and coniferous forest (Figure S2). When assigning a landscape cover classification for each site (1=urban parkland, 2=deciduous forest, 3=mixed forest, 4 = coniferous forest), we took into account the broader habitat surrounding the area and restricted land cover estimates to terrestrial habitats within 1 km of the sampling location. In addition to land cover, mean annual temperature (°C), mean annual precipitation (mm), and elevation (m) were included from the World BIOCLIM dataset (Version 2.1, Hijmans et al. 2005). We used a multivariate analysis of covariance (MANCOVA) in SPSS (Version 23.0) to compare habitat variation among the four chickadee species and to characterize the most important habitat variables for each species. In our analysis we included latitude as a covariate to account for environmental differences between northern and southern latitudes.

Analyses of admixture patterns across landscape matrices

We examined the effect of environmental variation on admixture among chickadees. To quantify how similar environments were between species pairs at each site, we calculated the difference in land cover, elevation, mean annual temperature, and mean annual precipitation. The difference represents the absolute difference between the mean environmental variables for each species for a given geographic area. Smaller values indicate more similar environments, while larger values indicate less similar environments. We then used linear mixed models to examine the relationship between admixture and environmental similarity. For this analysis, total admixture (the proportion of admixed individuals at each site) was our dependent variable, while environmental difference was the independent variable. We ran four different models (one for each variable) and included the species pair comparison as a covariate in our models to account for some species pairs being examined more frequently than others. These analyses were all run in R (R Core Team, 2020).

To test whether certain habitats have higher rates of admixture than others, we used a Chi-squared test in SPSS to test the null hypothesis that admixed individuals are distributed equally across all four of the habitat types. We examined the distribution of non-admixed and admixed individuals across the four habitat types (urban parkland, deciduous forest, mixed forest, and coniferous forest) and compared the observed frequency of non-admixed and admixed individuals across each habitat type to the expected frequencies. Finally, we extended this analysis to species pairs by comparing the distribution of admixed individuals among five species pairs, no admixed individuals were detected between Chestnut-backed and Mountain Chickadees, across the four habitat types. Again we used a Chi-squared test to compare the observed frequencies with the expected frequencies. It is important to note that sampling was skewed across habitats (χ^2=294.3, p<0.001) and among species (χ^2=686.1, p<0.001). Individuals from urban parkland and coniferous habitats exceeded expected frequencies, whereas individuals from mixed and deciduous habitats were lower than expected frequencies. Additionally more Black-capped Chickadees were sampled than the other three species.

Results

Rates of admixture among chickadees

We observed admixture for 63% (12 of 19) of the species pairs analyzed (Figure 3). On average 5% (± 1%) of individuals at each site showed evidence of admixture, although six species pair comparisons showed rates of admixture that exceeded this average.

Rates of admixture among species from different clades (6 ± 1%, N=11) were double the rate of admixture among species from the same clade (3 ± 1%, N=8; Black-capped vs Mountain Chickadees and Boreal vs Chestnut-backed Chickadees), although rates of admixture were not significantly different between these two groups (χ^2=1.0, p=0.31). Black-capped Chickadees (7 ± 1%; N=15) tended to exhibit higher rates of admixture with other species than Mountain (1 ± 1%; N=10), Chestnut-backed (2 ± 1%; N=6), and Boreal (3 ± 1%; N=7) Chickadees, although rates of admixture among the four species were not significantly different (χ^2=5.7, p=0.09).

When we examined the direction of admixture within species pairs, we found that admixed individuals with Black-capped Chickadee phenotypes were more frequent for our comparison of Black-capped vs Boreal Chickadee sites (χ^2=22.15, p<0.001) and for our comparison of Black-capped vs Chestnut-backed Chickadee sites (χ^2=3.88, p=0.05). For the remaining three species pair comparisons, there was no difference across sites for the frequency of admixed individuals between the species compared (Black-capped vs Mountain, Boreal and Chestnut-backed, and Boreal vs Mountain; χ^2=0.01 to 0.88, p>0.35).

Habitat differences among chickadees

Land cover and environmental data showed significant differences among the four chickadee species (Wilks' Lambda = 0.39, $F_{3, 434}$=39.9, p<0.001; Table 4; Figure 4). Mountain Chickadees occurred at higher elevation habitats (p<0.001), Boreal Chickadees occupied cooler habitats (p<0.001), Chestnut-backed Chickadees were found in wetter habitats (p<0.001). Black-capped Chickadees were found in deciduous forested habitats (p<0.001) compared to other chickadee species, which were found more often in mixed forested habitats.

Admixture rates among chickadee species were significantly different from zero (habitat: slope=0.07±0.02, t=2.9, p=0.01; elevation: slope=0.07±0.02, t=3.2, p=0.01; mean annual temperature: slope=0.05±0.02, t=2.1, p=0.05; mean annual precipitation: slope=0.06±0.02, t=2.6, p=0.02), but none of the environment dissimilarity variables were significant predictors (habitat: slope=-0.02±0.01, t=-1.32, p=0.21; elevation: slope=-0.01±0.01, t=-1.46, p=0.17; mean annual temperature: slope=-0.01±0.01, t=-0.44, p=0.67; mean annual precipitation: slope=-0.01±0.01, t=-0.87, p=040).

When we examined the habitats where admixed individuals were found, 50% (56 of 112) were found in urban or urban parkland areas, 22.3% were found in mixed forests, while 14.3% and 13.3% were found in coniferous and deciduous habitats (Figure 5a). Using a Chi-squared test, we were able to reject the null hypothesis that admixed individuals are equally distributed across the four habitat types (χ^2=11.7, p<0.01; Phi=0.07, p<0.01). Fewer admixed individuals were found in coniferous forests compared to expected frequencies, whereas the number of admixed individuals from mixed and urban parkland habitats exceeded frequencies expected by chance. When we expanded our analysis to account for species-pairs, again we found a significant difference in the distribution of admixture across the four habitat types (χ^2=55.0, $p<0.01$; ϕ = 0.7, $p<0.001$). For example, admixed Boreal and Black-capped, and Boreal and Chestnut-backed Chickadee individuals were primarily found in urban parkland habitats, while admixed Black-capped and Mountain, and Black-capped and Chestnut-backed Chickadee individuals were primarily found in mixed forest and coniferous forest habitat respectively. Given the low sample sizes for some of these within-species pair admixed individuals, these results should be viewed conservatively.

Discussion

Our study emphasizes how variable zones of admixture and hybridization are (Gill 2004, Lemmon and Juenger 2017); both in terms of the rate of admixture, and the geographic distribution of admixture. Admixture varied across habitats, and although the highest rates of admixture were observed in areas where species pairs occupied more similar habitats, habitat similarity was not a strong predictor of hybridization, as has been shown in other studies (Taylor et al. 2014, Bell and Irian 2019).

We documented admixture among five of the six chickadee species pairs examined; we did not detect any admixture between Mountain and Chestnut-backed Chickadees. The prevalence of admixture among chickadee species is not surprising given Justyn et al. (2020) found that among passerines, hybridization is most frequent in family Paridae. Mountain × Black-capped Chickadee and Boreal × Black-capped

Chickadee hybrids were previously documented by Grava et al. (2012) and Lait et al. (2012), but our study provides genetic evidence to suggest admixture occurs between Black-capped × Chestnut-backed, Boreal × Chestnut-backed, and Boreal × Mountain Chickadees and admixture rates vary based on geographic location. It is important to note that with exception of one individual (Boreal × Black-capped Chickadee hybrid, Lait et al. (2012), all of the birds were phenotypically identified to one species. Grava et al. (2012) noted all of the admixed chickadees identified in their study were phenotypically Mountain Chickadees. Justyn et al. (2020) used phenotypic analysis only and these results combined with our own suggest that hybridization may be higher than currently estimated as hybrids are not always phenotypically distinguishable or easy to detect based on phenotype alone (Ottenburghs and Slager 2020).

Admixture between species from the same clade (Black-capped and Mountain, and Boreal and Chestnut-backed Chickadees) was lower (based on the proportion of admixed individuals) than admixture between species from different clades. This result is opposite to what has been reported for other analyses of hybridization. In their review of hybridization among birds, Gholamhosseini et al. (2013) found that hybridization was more frequent between sister species than non-sister species for 25 of the 29 genera reviewed indicating hybridization is more common between sister species than non-sister species. That hybridization is common among non-sister species is not surprising, given the extensive evidence of hybridization between non-sister species across taxa (Dasmahaptra et al. 2007, Joseph et al. 2019, Tea et al. 2020). Although in our study admixture was higher between species from different clades, it is important to view these results in the context of the Black-capped Chickadee and Carolina Chickadee hybrid zone (both of these species are part of the same clade; Harris et al. 2014), where hybridization is frequent and well studied (Taylor et al. 2014, Wagner et al. 2020).

Our analyses detected a greater number of admixed individuals (Figure 5) for Boreal and Black-capped Chickadees; Boreal and Black-capped chickadees exhibit the greatest range overlap among the four species that we examined. Previous work has indicated that sympatry may be a better predictor of admixture. For example, Willis et al. (2014) found that rates of hybridization among wood warblers increased with sympatry. They speculated that sympatry may increase the opportunity for hybridization. By comparison Randler (2006) found that hybridization was more frequent between parapatric than sympatric species, although Randler notes that there are exceptions to this pattern and similar to Willis et al. (2014) states that sympatry may increase the potential for individuals to find mates (Tubaro and Lijtmaer 2002). Across taxa there is growing evidence that hybridization is common in natural populations among deeply divergent lineages and that sympatry may help to facilitate these events (Jasso- Martínez et al. 2018, Tea et al. 2020).

Among the sites we examined, the rates of admixture between species with more specialized habitat requirements (i.e., Mountain and Chestnut-backed Chickadees, Boreal and Mountain Chickadees, and Boreal and Chestnut-backed Chickadees) appear to be less frequent, but admixture and hybridization are likely to increase in response to decreases in landscapes heterogeneity (Seehausen et al. 2008). Over 70% (81 of 112) of individuals identified as admixed were found in urban parkland or mixed forest habitat. Alterations to habitat often through human-mediated processes have lead to the rise in hybridization between naturally co-occurring species, which under natural conditions would not interbreed (reviewed by Grabenstein and Taylor 2018). These habitat alterations may allow a more generalist species like the Black-capped Chickadee to expand into areas where it would not normally be found leading to increased mating opportunities with other chickadee species. For example, increased rates of deforestation and fragmentation have lead to increased rates of hybridization between European populations of wildcats (*Felis silvestris*; Hertwig et al. 2009) with domesticated cats. Similarly, alterations to vegetative structure around aquatic breeding habitats may increase hybridization between South American reed frogs (*Hyperolius thomensis* and *H. molleri*; Bell and Irian 2019). In central British Columbia, Grava et al. (2012) noted that although the majority of admixed Mountain x Black-capped Chickadees were found in forested areas, this area had been previously logged and subsequently replanted providing further support for the potential of habitat alterations to increase hybridization.

It is important to interpret the results of this study conservatively due to the low number of sites examined, as well as the uneven distribution of species examined at sites (both Boreal and Black-capped Chickadees were better represented than Chestnut-backed and Mountain Chickadees based on the number of samples and sites). A more comprehensive and balanced sampling approach would likely result in lower overall proportions of admixture between species pairs. Additionally our analysis of species pairs in areas where three or more parental species come into contact, did not allow us to fully account for individuals having ancestry from three or more parental populations. Therefore this study can be viewed as a first step in examining patterns of admixture among North American Parids. Moving forward next-generation sequencing should be used to examine and identify individuals with ancestry from three or more parental populations, as has been done to examine complex hybridization patterns for warblers (Toews et al. 2018). Despite these limitations, our study provides greater insights into the frequency of admixture in wild populations. Our examination of geographic patterns of admixture provides a framework for future studies of admixture and hybridization in the family Paridae. Future studies should incorporate morphological and phenotypic measurements to determine whether individuals identified as putative hybrids using genetic markers have intermediate morphological and plumage traits. Additionally the results of our investigation of the relationship between habitat, ecology, and genetic admixture can be used to test further questions about the relationship between these three variables. In particular studies conducted in areas where landscapes have been altered through human mediated events (Grabenstein and Taylor 2018) will provide greater insights into how common widely distributed species like chickadees are affected by anthropogenic changes.

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Table 1: List of the four North American chickadee species examined in this study, range sizes and a description of their preferred habitat. Range maps for each species are shown in Figure 1. Information on range size and habitat preferences were obtained from Bird Life International (2020).

Species	Scientific name	Range (10^6 km^2)	Habitat	Clade
Black-capped Chickadee	*Poecile atricapillus*	15	Deciduous, mixed, open parkland	Black-headed clade
Boreal Chickadee	*Poecile hundsonicus*	11.3	Coniferous forests	Brown-headed clade
Chestnut-backed Chickadee	*Poecile rufescens*	3.92	Coniferous forests, cedar-hemlock	Brown-headed clade
Mountain Chickadee	*Poecile gambeli*	4.36	Montane coniferous forests	Black-headed clade

Table 2: Total area (10^6 km^2) of range overlap among four North American Chickadees. Numbers in parenthesis represents the percentage of each species range that overlaps with the species it is compared with.

Species	**Black-capped Chickadee**	**Boreal Chickadee**	**Chestnut-backed Chickadee**	**Mountain Chickadee**
Black-capped Chickadee	-	7.19 (47%)	1.02 (7%)	2.79 (19%)
Boreal Chickadee	7.19 (63%)	-	0.36 (3%)	0.94 (8%)
Chestnut-backed Chickadee	1.02 (26%)	0.36 (9%)	-	0.74 (19%)
Mountain Chickadee	2.79 (64%)	0.94 (21%)	0.74 (17%)	-

Table 3: List of geographic areas where admixture analyses between species pairs were conducted. Species comparison shows the two chickadee species compared at the geographic area. N represents the sample size for species A and species B (based on phenotypic identification) compared at each site. Locations of sampling sites are shown in Figure 2. Habitat shows the most common habitat type for species A and Species B for each geographic area.

Geographic Area	Species Comparison	N	%admixed	Habitat
Alaska	Black-capped / Boreal	84/95	0.0%	urban parkland|deciduous
Alberta-Saskatchewan	Black-capped / Boreal	62/31	15.0%	mixed|mixed
Central British Columbia	Black-capped / Boreal	60/38	19.4%	urban parkland|coniferous
Eastern Canada	Black-capped / Boreal	142/71	13.6%	deciduous|coniferous
Newfoundland	Black-capped / Boreal	36/35	5.6%	urban parkland|deciduous
Alaska-northern British Columbia	Black-capped / Chestnut-backed	69/91	3.8%	urban parkland|coniferous
Washington	Black-capped / Chestnut-backed	28/44	2.8%	urban parkland|coniferous
Southeastern British Columbia	Black-capped / Chestnut-backed	30/54	4.8%	urban parkland|coniferous
Central British Columbia	Black-capped / Mountain	91/89	7.7%	mixed|mixed
Idaho-Montana	Black-capped / Mountain	80/43	0.8%	coniferous|coniferous
Southern Alberta	Black-capped / Mountain	52/23	12.0%	mixed|coniferous
Colorado-Utah	Black-capped / Mountain	51/58	0.0%	urban parkland|coniferous
Northeastern Oregon	Black-capped / Mountain	15/25	0.0%	urban parkland|coniferous
Southern Oregon	Black-capped / Mountain	15/26	0.0%	urban parkland|coniferous
Washington	Black-capped / Mountain	28/19	0.0%	urban parkland|coniferous
Northern British Columbia	Chestnut-backed / Mountain	91/89	0.0%	coniferous|coniferous
Washington	Chestnut-backed / Mountain	44/19	0.0%	coniferous|coniferous
Central British Columbia	Boreal / Mountain	38/33	1.4%	coniferous|coniferous
Alaska-northern British Columbia	Boreal / Chestnut-backed	99/91	5.3%	coniferous|coniferous

Table 4: Summary of habitat data for the four-chickadee species. N denotes the number of chickadee samples (based on phenotype) analyzed for this study across all species and population comparisons. For land cover, 1=urban parkland habitat, 2=deciduous forest, 3=mixed forest, 4=coniferous forest. Values represent the mean ± se.

Species	N	Land Cover	Elevation (m)	Mean Annual Temperature (°C)	Mean Annual Precipitation (mm)
Black-capped Chickadee	780	2.0 ± 0.1	744 ± 37	3.6 ± 0.2	732 ± 25
Boreal Chickadee	280	2.5 ± 0.1	639 ± 54	1.9 ± 0.2	773 ± 36
Chestnut-backed Chickadee	168	2.8 ± 0.2	652 ± 102	5.7 ± 0.4	1744 ± 68
Mountain Chickadee	200	2.6 ± 0.2	1515 ± 82	2.2 ± 0.3	476 ± 47

Figure 1: Maps show the distribution range a) Black-capped Chickadees, b) Boreal Chickadees, c) Chestnut-backed Chickadees, and d) Mountain Chickadees. Range maps were recreated using data downloaded from BirdLife International and Handbook of the Birds of the World (2016).

Figure 2: Maps show the areas of range overlap between a) Black-capped and Boreal Chickadees, b) Black-capped and Chestnut-backed Chickadees, c) Black-capped and Mountain Chickadees, d) Boreal and Chestnut-backed Chickadees, e) Boreal and Mountain Chickadees, and f) Chestnut-backed and Mountain Chickadees. Black circles represent sampling locations in areas of sympatry, while white dots represent the allopatric sampling locations.

Figure 3: Plots showing rates of admixture among four North American chickadee species: Black-capped (*Poecile atricapillus;* red), Boreal (*Poecile hudsonicus*; green), Chestnut-backed (*Poecile rufescens*; blue), and Mountain (*Poecile gambeli;* purple) Chickadees. The location of each species pair comparison is listed above the plot. The assignment probability to species A (Y axis) and species B (X axis) from STRUCTURE at K=2 is shown for all comparisons. The black line represents the probability of an individual equally assigning to species A and species B; all values above the line represent individuals assigned to species A, while values below this line represent individuals assigned to species B.

Figure 4: Plots showing habitat and environmental differences among four North American chickadee species Black-capped (*Poecile atricapillus;* red), Boreal (*Poecile hudsonicus*; green), Chestnut-backed (*Poecile rufescens*; blue), and Mountain (*Poecile gambeli;* purple) Chickadee. For land cover, 1=urban parkland, 2=deciduous forest, 3=mixed forest, 4=coniferous forest. Error bars represent the upper and lower 95% confidence intervals.

Figure 5: Top shows the distribution (based on total percent) of admixed individuals (n=112) across the four habitat types: urban parkland (red), coniferous forests (green), deciduous forests (yellow), and mixed forests (pink). Bottom shows the breakdown of admixed individuals within each species pair across the four habitat types. As no putative Mountain and Chestnut-backed chickadees were detected, they are not shown on the figure.

Figure S1: a) to f) show pairwise comparisons of genetic differentiation for allopatric Chickadee populations using Principal Coordinate analyses; g) shows genetic differentiation for all four chickadee species combined. Red circles represent Black-capped Chickadees, blue triangles represent Chestnut-backed Chickadees, green squares represent Boreal Chickadees, and purple diamonds represent Mountain Chickadees.

Figure S2: Examples of three landscape matrices examined for this study. Land cover is classified into four categories urban parkland (red), deciduous forest (orange), mixed forested habitat (yellow), and coniferous habitat (green); blue represents water or wetland habitat. A) A landscape (Newfoundland) where Boreal Chickadees (*Poecile* *hudsonicus*) and Black-capped Chickadees (*Poecile atricapillus*) come into contact; B) a landscape (central British Columbia) where Mountain Chickadees (*Poecile gambeli*) and Black-capped Chickadees come into contact; and C) a landscape (southeast British Columbia) where Chestnut-backed Chickadees (*Poecile rufescens*) and Black-capped Chickadees come into contact.