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Research Article

Migratory Flyways May Affect Population Structure in Double-Crested Cormorants

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ABSTRACT Double-crested cormorants (*Phalacrocorax auritus*) recovered from a demographic bottleneck so well that they are now considered a nuisance species at breeding and wintering grounds across the United States and Canada. Management of this species could be improved by refining genetic population boundaries and assigning individuals to their natal population. Further, recent radio-telemetry data suggest the existence of Interior and Atlantic migratory flyways, which could reduce gene flow and result in substantial genetic isolation. In this study, we used 1,784 individuals collected across the eastern United States, a large panel of microsatellite markers developed for this species, and individuals banded as chicks and recaptured as adults to explore the effects of migratory flyways on population structure, quantify the genetic effects of demographic bottlenecks, and determine whether individuals could be assigned to their natal population based on genotype. We found evidence for genetic population division only along migratory flyways, no evidence of genetic bottlenecks, and mixed effectiveness of assignment tests. Our population structure findings suggest that gene flow is high across large scales; for example, individuals from New York, Minnesota, and Alabama are all in panmixia. We also found that traditional subspecies ranges may not be valid because >1 subspecies was present in single genetic populations. The lack of evidence for genetic bottlenecks also likely underscores the vagility of this species, suggesting that even during demographic bottlenecks, populations were not isolated from allelic exchange. Finally, the failure of assignment tests to consistently perform is likely due in part to imperfect *a priori* sampling of Atlantic and Interior chicks and the high vagility of adults. We conclude that the demographic bottleneck is not likely to have reduced genetic diversity, and that assignment tests remain unreliable for this species. We recommend double-crested cormorants be managed by flyway. Further development of genomic resources in this species could improve population subdivision resolution, improve assignment tests, and reveal further information on demographic histories. © 2020 The Wildlife Society.

KEY WORDS bottleneck, migration, North America, pest species, *Phalacrocorax auritus*, populations, subspecies.

Double-crested cormorants (*Phalacrocorax auritus*; cormorants) are large, piscivorous, colonial waterbirds with a dynamic demographic history of declines and recoveries in North America (Dorr et al. 2014*b*). Cormorants were abundant on the continent when Europeans first arrived (Wires and Cuthbert 2006). By the late nineteenth and early twentieth centuries, however, the abundance of cormorants across much of North America had declined because of overhunting and persecution by humans (Wires and Cuthbert 2006). Cormorants experienced a short-lived recovery in the 1920s to 1940s, which was likely reversed by widespread use of dichlorodiphenyltrichloroethane (DDT), habitat change, and legal and illegal control activities (Wires and Cuthbert 2006, Dorr et al. 2014*b*). Cormorants reached population lows in the 1970s, when fewer than 200 nesting pairs were recorded in the Great Lakes (Weseloh et al. 2002).

Cormorants have undergone an astonishing population recovery since the 1970s. A combination of adaptation to anthropogenic change (aquaculture, construction of reservoirs), pollution reduction, and regulatory protection facilitated a rebound from around 200 nesting pairs in the 1970s to approximately 115,000 nesting pairs in 2000 in the Great Lakes region alone (Weseloh et al. 2002, Dorr and Fielder 2017). Because of its contemporary abundance and widespread distribution, the double-crested cormorant is now often associated with human-wildlife conflicts (Dorr et al. 2014*b*). These conflicts, particularly with respect to commercial and natural resources such as aquaculture and sport fisheries, have resulted in often-controversial management of cormorants as

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a nuisance wildlife species (Dorr and Fielder 2017). One question central to this contentious issue is whether cormorants should be classified into subspecies, and, if so, how those subspecies should be regulated and managed (Mercer et al. 2013, Sheehan et al. 2017).

Many avian species, including the double-crested cormorant, experienced severe bottlenecks in the nineteenth and twentieth centuries (Grier 1982, Fry 1995). Historical demographic bottlenecks can leave genetic signatures on extant population allele frequencies (Luikart et al. 1998), although demographic bottlenecks are sometimes not detected using genetic data (e.g., Busch et al. 2007), especially if a limited number of loci are examined (Peery et al. 2012). Nonetheless, species that have undergone bottlenecks across their range often exhibit evidence of genetic differentiation over geographic distances, which is enhanced by genetic drift within populations and eroded by genetic exchange among populations.

Today, the use of various types of genetic assignment tests, often using Bayesian approaches with or without input on geographic location of sample collection, can assist wildlife managers with delineation of population boundaries and provide evidence of historical genetic exchange among populations. Genetic assignment tests attempt to assign the genotype of an individual to a population of origin, based on the genotype frequencies of the putative populations of origin. Over the past decade, such genetic tools have become increasingly important for delineation of population management units for game, non-game, and pest species. For example, Robertson and Gemmell (2004) employed assignment tests on multiple populations of black rats (*Rattus rattus*) on the island of South Georgia to determine that the successful movement of individuals among populations was rare, and that the elimination of the populations could proceed serially without risk of recolonization.

The delineation of population boundaries is a primary, preliminary goal in the management of species of conservation concern, both those in decline and those considered pests (Manel et al. 2003, Kirk et al. 2013), and several attempts have been made to delineate cormorant population boundaries. Green et al. (2006) used microsatellite loci developed for the great cormorant (*P. carbo*) to test for population structure in 179 individuals sampled at 9 sites. Treating them as *a priori* populations, they reported small but significant population differentiation as defined by F_{ST} values, no evidence for isolation by genetic distance, no evidence for bottlenecks, and that <25% of birds were successfully assigned to their *a priori* sampling site. They concluded that wintering birds cannot successfully be assigned to their breeding colonies; however, they used only 5 loci, which is likely an insufficient number to correctly assign individuals to populations (Paetkau et al. 2004). Mercer et al. (2013) used 8 microsatellite loci, 6 of which were developed specifically for double-crested cormorants, and 2 mtDNA loci to evaluate 409 individuals from 23 breeding colony sites, including birds from the southwestern United States and Alaska. They reported evidence for 3 population clusters across much of the species' range and a pattern of genetic isolation by geographic distance. In light of recent work demonstrating behavioral differences in migration patterns of double-crested cormorants (Guillaumet et al. 2011), however, population structure should be reassessed.

In the current study we build upon and expand earlier efforts to characterize the population genetics of double-crested cormorants by exploring the influence of migratory flyways on population structure, testing for genetic signatures of demographic bottlenecks, and testing the utility of genetic assignment tests of individuals to their natal population. Our first hypothesis is that there is significant population differentiation in double-crested cormorants in eastern North America between Mississippi and Atlantic flyways because gene flow is reduced between them. Our second hypothesis is that because double-crested cormorants are natally philopatric, assignment tests can identify the breeding colony of origin for individuals captured in southern (wintering and feeding) flocks.

STUDY AREA

We conducted our study on or near double-crested cormorant breeding colonies in the eastern United States in Alabama, Michigan, Minnesota, Mississippi, New York, and Vermont and at night roost locations and aquaculture facilities on the wintering grounds in Alabama, Arkansas, and Mississippi in the southeastern United States (Fig. 1). Collections of birds from the 1.9 million-km^2 study area (47.443 N 94.745 W; 45.045 N 72.946 W; 32.180 N 85.116 W; 32.469 N 92.272 W) occurred from 2004 to 2007, and in April 2010 (Dorr et al. 2016*a*).

Northern breeding colonies located in Lake Huron, Michigan; Leech Lake, Minnesota; Lake Ontario, New York; and Lake Champlain, Vermont are on islands or portions of islands typically <10 ha in large lakes, at elevations that range from 30 m to 400 m above sea level. Cormorants often nest on the ground (substrates of rock, sand, or soil) on these islands because they are devoid of vegetation but will nest in live trees and standing snags when present in these locations. Temperatures during the breeding season ranged from 4°C to 7°C in April to 19°C to 22°C in July with a warm-summer humid continental climate. Annual precipitation ranged from 10 cm in Minnesota to 55 cm in Vermont. This area is part of the temperate broadleaf and mixed forest biome, and includes Eastern Great Lakes lowland forests and Western Great Lakes forests ecoregions. Dominant fauna from collection areas included co-nesting species such as gulls (*Larus* spp.), terns (*Sterna* spp.), and American white pelican (*Pelecanus erythrorhynchos*). Dominant vegetation included pine (*Pinus* spp.), oak (*Quercus* spp.), paper birch (*Betula papyrifera*), and aspen (*Populus tremuloides*).

Southern breeding colonies located in Lake Guntersville, Alabama and the Delta region of Mississippi are located in swamps, lakes and rivers. Southern breeding cormorants nest in trees on islands in large lakes and rivers, or in bald cypress (*Taxodium distichum*) and tupelo gum (*Nyssa sylvatica*) trees in standing water and swamps, at elevations that range from 45 m to 180 m above sea level. The wintering grounds included commercial aquaculture farms in Arkansas and Mississippi; night roost locations occurred

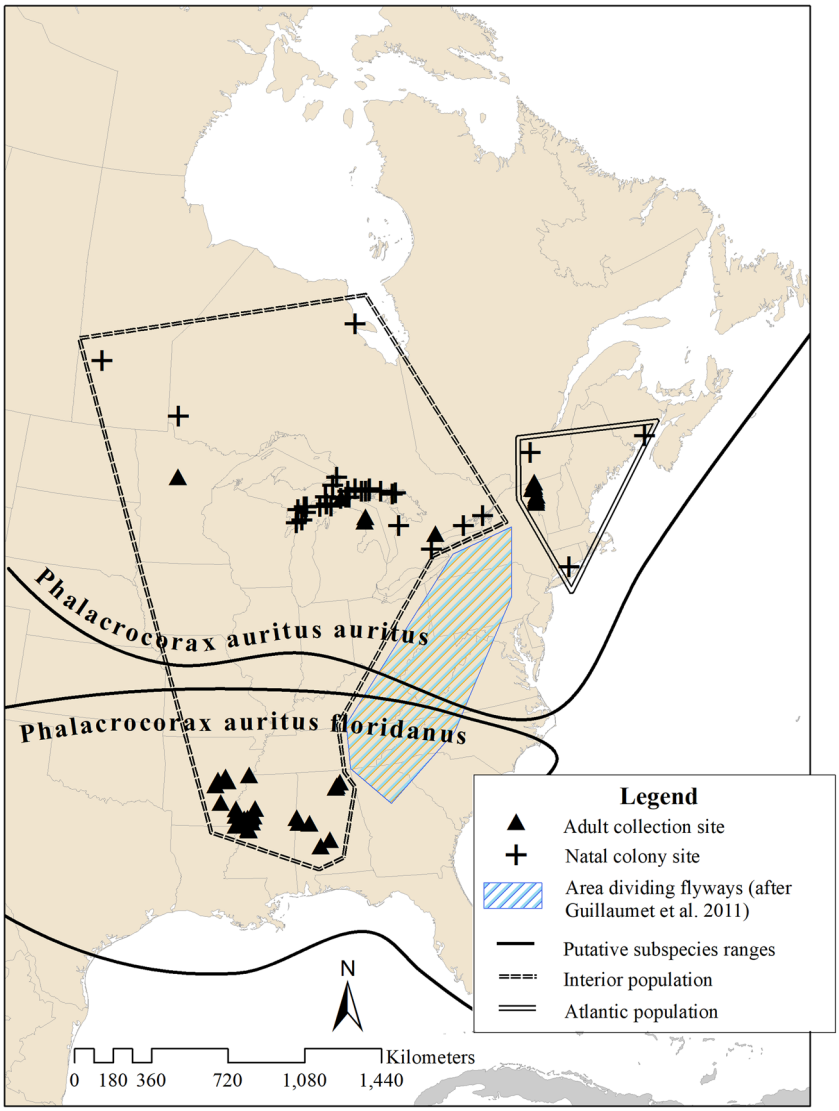

Figure 1. Adult collection and natal colony locations of double-crested cormorants in eastern North America, 2004–2010. Triangles (▲) represent locations where individuals ($n = 1{,}775$) were collected from breeding colonies, feeding flocks, and night roosts on the wintering grounds. Crosses (+) represent natal colony locations of a subset of sampled adult birds that were fitted with a leg band as chicks. Area dividing Atlantic and Mississippi flyways is per Guillaumet et al. (2011). The corresponding Atlantic and Interior populations, as identified in the current study, are outlined.

in trees along rivers and lakes in Alabama, Arkansas, and Mississippi at elevations of 40 m to 70 m above sea level (Dorr et al. 2014*a*). Temperatures in the southern breeding colonies and wintering grounds ranged from 6°C to 8°C in January and 27°C to 28°C in July and the area is considered a humid subtropical climate. Annual precipitation ranged from 55 cm in Arkansas to 66 cm in Alabama. The area included Southeastern mixed forests (oak [*Quercus* spp.]-hickory [*Carya* spp.] forests and mesic mixed hardwood forests) and Mississippi lowland forests. Dominant vegetation included oak, hickory, pine (*Pinus* spp.), American beech (*Fagus grandifolia*), tulip tree (*Liriodendron tulipifera*), American hornbeam (*Carpinus caroliniana*), bald cypress, water tupelo (*Nyssa aquatica*), and black willow (*Salix nigra*). Dominant fauna from collection areas included waterbirds such as great blue heron (*Ardea herodias*), great egret (*Ardea alba*), and American white pelican; waterfowl (Anatidae); North American beaver (*Castor canadensis*); and muskrat (*Ondatra zibethicus*).

METHODS

In this study, we first employ a larger panel of microsatellite loci to evaluate a much larger sample of individuals across populations than earlier studies. Power to resolve metrics typically employed in population genetic analyses likely increases with locus number (Pritchard and Rosenberg 1999, Evanno et al. 2005) and large sample sizes can improve the ability of algorithms to improve population genetics parameter estimates (Hale et al. 2012). Second, we developed a microsatellite panel using a subsample of individuals from the populations we evaluated (Fike et al. 2009). Microsatellite loci developed from the DNA of a species of interest, as is the case in our study of double-crested cormorants, tend to have higher allelic richness and statistical

power than do homologous loci developed in related species (FitzSimmons et al. 1995, Forbes et al. 1995). Third, our sample set contained banded individuals. This allows us to evaluate genotypes in the contexts of both their natal (banding) and adult capture locations, an approach that can shed light on the natural history of this vagile species (Brownie et al. 1985), especially when coupled with genetic analyses (Duckworth and Badyaev 2007). Finally, recent work by Guillaumet et al. (2011), using extensive data from modern telemetry methods, indicated that there are 2 migratory pathways employed by most cormorants that nest in the Great Lakes region. This last development led us to expect that the geographic delineation of double-crested cormorant populations in the central United States would follow flyway patterns (Ely et al. 2017).

We salvaged tissue samples (skin or muscle) from cormorants ($n = 1{,}775$) collected by federal agency staff as part of control efforts. We obtained muscle tissue samples from a subset of these individuals ($n = 262$) that were previously banded as chicks and therefore had natal colony information for where these cormorants hatched (Fig. 1). We froze tissues until analyses. Birds were salvaged under United States Department of the Interior, United States Geological Survey, Federal Bird Marking and Salvage Permit (20873) and salvage was approved by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center's Institutional Animal Care and Use Committee under Quality Assurance Protocol QA-1398.

To extract DNA, we used a standard proteinase K and phenol-chloroform-isoamyl alcohol DNA extraction method (Sambrook and Russell 2001). We resuspended DNA in 50 μL of TLE (10 mM tris, 0.1 mM EDTA, pH 8.0), and quantified sample concentrations on a NanoDrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). We used 10 microsatellite loci previously developed for double-crested cormorants (Fike et al. 2009) multiplexed into 2 reactions. We analyzed all polymerase chain reaction (PCR) products on an automatic sequencer (ABI 3730XL, Applied Biosystems, Foster City, CA, USA). We scored genotypes automatically followed by manual calling using GeneMapper (version 3.7; Applied Biosystems). For quality control we reamplified approximately 10% of all reactions in addition to any genotypes for which there were conflicting calls.

We used CERVUS (version 3.0.3; Kalinowski et al. 2007) to calculate the probability of identity and to guard against accidentally duplicated samples (e.g., by human error in sample labeling) by identifying and discarding them from further analyses. We used the R package POPGENREPORT (Adamack and Gruber 2014) to evaluate the genotype data, including allelic richness, expected and observed heterozygosity, departures from Hardy-Weinberg equilibrium (HWE), and presence of null alleles.

To evaluate our first hypothesis, we used the Bayesian program STRUCTURE (version 2.3.2; Pritchard et al. 2000) to determine if population genetic structure exists among all samples. We ran STRUCTURE 10 times for each number of putative populations (k) from 1 to 10 using the correlated allele frequency model (Latch et al. 2011), and conducted each run for 500,000 iterations with a 100,000-iteration burn-in. We then used 3 methods to estimate the correct number of k from the STRUCTURE output (Pritchard et al. 2000, Evanno et al. 2005, Janes et al. 2017). First, we used STRUCTURE HARVESTER (version 0.6.92; Earl and vonHoldt 2012) to infer the best k value according to the Δk method of Evanno et al. (2005). Second, we evaluated the posterior probability of each value of k (Pritchard et al. 2000). Third, we examined barplots of the q values, the proportion of each individual's genotype assigned to each of k populations (Pritchard et al. 2000) assigned by STRUCTURE. We used CLUMPP (Jakobsson and Rosenberg 2007) to combine the output from the 10 multiple runs STRUCTURE produces for each value of k, and DISTRUCT (Rosenberg 2004) to visualize the barplots as implemented in CLUMPAK online (Kopelman et al. 2015). For any result not $k = 1$, we used a hierarchical analysis by applying these same methods to each cluster to attempt to detect any substructuring (Vähä et al. 2007).

Allele frequency shifts can accompany population expansions (Kimmel et al. 1998), which may have occurred in cormorants as they recovered from a demographic bottleneck (Dorr and Fielder 2017). To test whether allele frequencies had shifted over the time frame of our study, we selected 2 banding sites (Spider Island, WI and St. Martin's Shoal, Lake Huron, MI) where sufficient numbers of chicks were banded from the same location across the study period and performed STRUCTURE analyses as described above.

We also used the populations as assigned by STRUCTURE to test for the presence of cormorant population bottlenecks in the program BOTTLENECK (Piry et al. 1999). We used the default settings to conduct Wilcoxon tests (Luikart et al. 1998) with a 2-phased model of mutation because this is the most appropriate for microsatellite loci (Ellegren 2000). We also used a mode-shift test (Luikart and Cornuet 1998), which does not require a mutation model.

Further, we used GENALEX (version 6.51; Peakall and Smouse 2012) to quantify genetic distance between populations (R_{ST}) and to implement an analysis of molecular variance (AMOVA) to quantify variance within individuals, among individuals, and among populations (as determined by STRUCTURE analyses). We used 999 permutations to assess significance. We also tested for genetic isolation by geographic distance across all samples as assessed using 999 permutations.

To address our second hypothesis, we used our subset of samples that came from individuals banded as chicks before being collected as adults to determine if individuals could be correctly assigned to their natal region based on their genotype. To investigate the incidence of intra-population mixing (and therefore potentially interbreeding), we employed 2 assignment tests on the recaptured adult samples. First, we used the frequency-based estimator of Paetkau et al. (1995) within the program GENECLASS2 (Piry et al. 2004) to assign a probability of membership to

Table 1. Summary statistics of 10 microsatellite loci (Fike et al. 2009) for double-crested cormorants from eastern North America, 2004–2010. A_R = allelic richness; H_E = expected heterozygosity, H_O = observed heterozygosity, HWE = Hardy-Weinberg equilibrium.

Locus	A_R	H_E	H_O	HWE (Interior)	HWE (Atlantic)	Probability of null alleles
COR01	21	0.779	0.772	0.000	0.020[a]	0.004
COR03	21	0.886	0.854	0.000	0.000[a]	0.017[b]
COR05	10	0.742	0.667	0.000	0.088	0.044[b]
COR06	16	0.884	0.875	0.376	0.133	0.005
COR15	6	0.559	0.541	0.596	0.997	0.011
COR19	13	0.746	0.726	0.641	0.247	0.012[b]
COR28	10	0.695	0.678	0.982	0.838	0.010
COR30	11	0.740	0.753	0.007	0.237	−0.007
COR38	6	0.611	0.599	0.200	0.966	0.008
COR45	6	0.331	0.335	0.120	0.507	−0.003
$\bar{x}$	12.0	0.697	0.680	0.292	0.403	0.004

[a] Significant departure from HWE.
[b] Significantly different from zero.

each unknown individual to the populations defined by STRUCTURE. We used all resampled adults, banded as chicks, as the training set and genotypes from all others as the unknowns. We assigned the resampled adults to a known population based on their geographic location of banding and used a machine-learning approach in the R package ASSIGNPOP (version 1.1.4; Chen et al. 2018) to attempt to assign individuals to populations. We used the same training and unknowns data, and selected randomForest as the model for the assignment of unknowns (Sylvester et al. 2018).

RESULTS

We collected 1,784 tissue samples. Each of these was successfully genotyped for ≥9 of 10 loci (Table 1). We removed 9 samples because of accidental resampling of previous captures or genotyping error, leaving 1,775 individuals for downstream analyses, including 162 from individuals banded as chicks and resampled as adults (Fig. 1). We calculated the probability of exclusion to be 8.84×10^{-11}.

The Δk method showed the highest support for $k = 2$ (Fig. S1, available online in Supporting Information) with a relatively high value (~250; this method cannot distinguish between $k = 1$ and $k = 2$; Janes et al. 2017). The posterior probability for $k = 2$ was greater than $k = 1$ (Fig. S2, available online in Supporting Information) and the STRUCTURE barplot also provided support for $k = 2$ (Fig. 2; Fig. S3, available online in Supporting Information). We judged that the strength of the $\Delta k = 2$ spike, the higher posterior probability for $k = 2$, and the difference in genotype admixture visible in the boxplot all support a conclusion that $k = 2$ for population structuring within our cormorant samples.

These 2 clusters are composed of the Vermont and New York samples (i.e., Atlantic population) and all other samples (i.e., Interior population). We subjected genotypes from these 2 clusters to subsequent hierarchical STRUCTURE analyses, using only samples assigned to the Interior or Atlantic populations but found no evidence for substructuring in either population. We tested for temporal shifts in allele frequencies. We analyzed the genotypes of 30 individuals banded as chicks at St. Martin's Shoal: 15 from June 1987 to July 1991 and 15 from July 1995 to June 2002. We found no evidence for any population structure using the delineation method as described above. We analyzed the genotypes of 50 individuals banded as chicks at Spider Island, Wisconsin: 25 from June 1989 to July 2001 and 25 from July 2003 to June 2006. We found no evidence for population delineation within these samples. In addition, we found no evidence for population bottlenecks in our analyses of banded cormorants in either population using either method we employed ($P < 0.05$ and normal L-shaped distribution of allele frequencies).

For the R_{ST} and AMOVA analyses, we divided genotypes into Interior and Atlantic populations. We calculated an R_{ST} of 0.02 ($P = 0.001$) and 1.99% of variation was between populations, 87.79% was among individuals, and 10.22% was within individuals. There was significant genetic isolation by geographic distance ($r = 0.020$, $P = 0.026$) in our data.

We collected 159 banded birds in areas encompassed by the Interior population (as determined by their banding in areas assigned to Interior population in the Bayesian analysis, above), and we collected 3 in areas included in the Atlantic (1 each on Long Island, Bay of Fundy, and

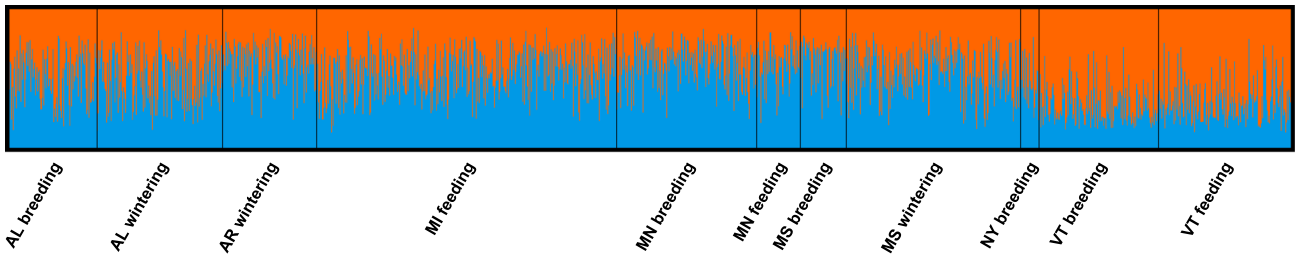

Figure 2. Barplot for $k = 2$ for all samples of double-crested cormorant in eastern North America, 2004–2010. Only $k = 2$ is presented because this is the most likely number of populations present in our samples. Each individual is represented by as single column and individuals are grouped by state and season of collection (e.g., AL breeding). The proportion of each individual's genotype originating from each population is indicated by the 2 colors. The relatively higher average proportion of genotypes colored blue in the AL breeding through MS wintering collections contrasts with the relatively higher proportion of genotypes colored orange in the NY breeding through VT feeding collections, suggesting 2 populations overall, though the differences are not substantial. Samples were collected from breeding colonies (breeding), wintering flocks (wintering), and flocks feeding near breeding colonies but not necessarily consisting of breeding birds (feeding).

Lac St. Pierre, Quebec, Canada). We used these data as the training data and all individuals of unknown natal location as the queries. The combined percent of individuals correctly assigned to the population from which they were collected was 67.4% and 76.0% for the frequency-based and machine-learning methods, respectively. The percent correctly assigned was greater for the Interior population (frequency-based: 76.3%, machine-learning: 98.5%) than for the Atlantic population (frequency-based: 38.2%, machine-learning: 2.0%).

DISCUSSION

Double-crested cormorants are piscivorous and locally abundant, and are therefore of particular concern for aquaculture in the southeastern United States where they overwinter (Duffy 1995). Dispersal and culling have been key management tools (Dorr et al. 2016*b*) but could be improved if problematic wintering populations could be tied to specific breeding colonies because cormorants show significant colony fidelity (Duffy 1995, Dorr et al. 2014*b*). Using the advantages in this descriptive study as identified above, assignment tests potentially could be used to associate adults with breeding grounds (Rollins et al. 2009) and make control efforts more efficient.

Our findings suggest that double-crested cormorants in our study are genetically diverse and weakly divided into 2 populations. Cormorants experienced a demographic bottleneck in the twentieth century, but this appears not to have left a genetic signature because mean allelic richness is 12.0, mean observed heterozygosity is 0.680, and we found no evidence for genetic bottlenecks. In addition, the only population structuring we detected with these data is at the region-wide scale, as evidence by both Bayesian and AMOVA analyses, and is a product of genetic isolation by distance. Further, this structuring corresponds to a division between the Mississippi and Atlantic flyways, as suggested by direct observation (Guillaumet et al. 2011). Substantial gene flow still exists across this divide, however, as suggested by satellite telemetry (Guillaumet et al. 2011) and by the relatively weak signal of population differentiation ($R_{ST} = 0.02$, $P = 0.001$) and relatively weak signal of genetic isolation by distance ($r = 0.020$, $P = 0.026$) in the current work (Fig. 2).

The high genetic diversity found in these samples has 2 major implications, one historical and one projected. In the former, high diversity suggests that the demographic bottleneck the species experienced in the twentieth century (Hatch 1995) was not severe enough to have left a clear genetic signature, or at least it is not yet evident. This is likely due to a relatively wide bottleneck, rapid demographic recovery (Brown et al. 2007) due to relaxation of pollution pressures, increases in available resources (e.g., aquaculture), and government protection (Weseloh et al. 1995, Dorr et al. 2014*b*). The latter implication of high genetic diversity is that evolutionary potential likely remains high. For example, following release from the demographic bottleneck mentioned above, cormorants have recolonized their historical range and then expanded this range (Hobson et al. 1989, Post 1998). This range expansion, likely in response to increased anthropogenic resources such as aquaculture and human-made lakes, may suggest the exploitation of evolutionary potential (Griffith and Watson 2006, Lavergne and Molofsky 2007). Alternatively, this may be due to native phenotypic plasticity (Grémillet and Charmantier 2010).

Our data suggest that the individuals sampled here can be divided into 2 weakly defined populations. The Atlantic population is represented by individuals from the adjacent states of Vermont and New York (and possibly farther north and east), and the Interior population included birds sampled in Alabama, Mississippi, Arkansas, Michigan, and Minnesota. We did not detect any substructuring of either population, suggesting that gene flow is high at the flyway scale and that management of cormorants should likewise be managed at this regional scale. Cormorants have historically been managed at the state or local level (50 CFR 21.48, U.S. Fish and Wildlife Service [USFWS] 2014) and smaller scales (50 CFR 21.47, USFWS 2014). For example, the USFWS issues depredation permits to state or local agencies or to private aquaculture facilities, but does not have a policy to formally manage cormorants at a flyway level. Our results contrast those of Waits et al. (2003), who reported no evidence of structure. Our results echo those of Green et al. (2006), who reported substantial gene flow among populations from North Dakota to Lake Champlain, and Mercer et al. (2013), who reported substantial gene flow from Alberta to Nova Scotia, Canada, and Minnesota to Massachusetts, USA. Together, these studies suggest that although gene flow is not unlimited (e.g., R_{ST} was significant between Interior and Atlantic populations), it occurs widely at the regional level.

Our results also imply that the apparent line of demarcation between the 2 populations cuts across subspecies designations. The geographic designations that follow Dorst and Mougin (1979) and Watson et al. (1991) suggest that the cormorants breeding in Arkansas, Mississippi, and Alabama could be from expansions of the *P. a. floridanus* range (Sheehan et al. 2017), whereas our samples suggest they are not distinct from cormorants originating from Minnesota, Michigan, New York, and Vermont, and therefore are more likely from the *P. a. auritus* population (both subspecies are referred to by the same common name). Further, we collected samples from both wintering flocks (putatively *P. a. auritus*) and breeding colonies (putatively *P. a. floridanus*) in Mississippi and for the first time in Alabama (Fig. 1), all of which clustered with the Interior *P. a. auritus* population.

Subspecies designations imply limited local gene flow, which would be expected to result in a genetic break between these 2 groups of samples. Instead, our data indicate that gene flow is high between our samples from the Arkansas-Mississippi-Alabama area (putatively *P. a. floridanus*) and the Minnesota-Michigan area (putatively *P. a. auritus*) but more limited between our Minnesota-Michigan area (putatively *P. a. auritus*) samples and our New York-Vermont area (also putatively *P. a. auritus*). This suggests that southern breeding cormorants from Arkansas

eastward to Alabama appear to be *P. a. auritus* and not an expansion of the range of *P. a. floridanus*. Similar results were reported by Waits et al. (2003), Green et al. (2006), and Mercer et al. (2013). Thus, all of the genetic data currently available for the double-crested cormorant suggest that the subspecies designations for cormorants may need revision. Further evaluation of genomic differences between cormorants originating from the Mississippi Flyway and those from the Atlantic Flyway would be helpful with regard to possible subspecies designations, as would further evaluation of breeding cormorants in other southern states (e.g., GA, FL).

The results of the assignment tests must be interpreted with caution. They correctly assigned many samples taken in the geographic location of the Interior population to the Interior genetic population (76.3% and 98.5%), but Atlantic samples were not frequently assigned to the Atlantic genetic population (38.2% and 2.0%). This is likely due to a combination of 3 factors. The first is that our dataset does not include training samples from all natal populations from which individuals might have originated (Paetkau et al. 2004). The second factor is that the vagility of this species makes it likely that some individuals of unknown population origin did not originate in the population from which they were captured. The third (and likely strongest) factor is that in our dataset there are only 3 individuals of known natal location in the Atlantic region. Unbalanced training dataset size is known to bias results towards the population(s) with larger sample sizes (Wang 2017). Despite these limitations, we present the data here to underscore the need for further field sampling because the ability to assign individual birds to population of origin will be a powerful management tool.

These data suggest genetic structuring of the Interior and Atlantic populations of double-crested cormorants and is supported by previously published migration and movement data (Guillaumet et al. 2011). Given the approximate geographic boundaries of these populations in this study, an increase in the dataset for the Atlantic population would improve assignment tests. Lastly, the further development of genetic resources for cormorants will likely enable additional assignment tests, further our understanding of population genetic processes such as flyway connectivity, and refine subspecies designations.

MANAGEMENT IMPLICATIONS

The piscivorous double-crested cormorant is frequently in conflict with humans because of their effects on commercial and natural fisheries and as such is subject to management, including lethal control by state, federal, and tribal agencies. Our data suggest that flyway-based management of cormorants east of the Rocky Mountains is supported by weak genetic structuring of the Interior and Atlantic populations and evidence of a permeable migratory divide between these flyways in cormorants. These data also indicate that double-crested cormorants that breed in Alabama are part of the interior *P. a. auritus* population and not the *P. a. floridanus* population of cormorants, which helps to clarify the management status of cormorants breeding in southeastern states. Currently, cormorant management is undergoing substantial policy revision including overall take levels. Given the data presented here, management-related take may be considered separately for the Mississippi and Atlantic flyways and cormorants breeding in southern states from Arkansas east to Mississippi should be considered as part of the *P. a. auritus* population.

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