




## Research Article

# Population Genetics of a Translocated Population of Mottled Ducks and Allies

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**ABSTRACT** Translocating species is an important management tool to establish or expand the range of species. Success of translocations requires an understanding of potential consequences, including whether a sufficient number of individuals were used to minimize founder effects and if interspecific hybridization poses a threat. We provide an updated and comprehensive genetic assessment of a 1970s–1980s translocation and now established mottled duck (*Anas fulvigula*) population in South Carolina, USA. In addition to examining the population genetics of these mottled ducks, we simulated expected genetic assignments for generational hybrids (F1–F10), permitting formal purity assignment across samples to identify true hybrids and establish hybridization rates. In addition to wild mallards (*A. platyrhynchos*), we tested for presence of hybrids with migrant American black ducks (*A. rubripes*) and released domestic game-farm mallards (*A. p. domesticus*). We used wild reference populations of North American mallard-like ducks and sampled game-farm mallards from 2 sites in South Carolina that could potentially interbreed with mottled ducks. Despite 2 different subspecies of mottled duck (Florida [*A. f. fulvigula*] and the Western Gulf Coast [*A. f. maculatus*]) used in original translocations, we determined the gene pool of the Western Gulf Coast mottled duck was overwhelmingly represented in South Carolina's current population. We found no evidence of founder effects or inbreeding and concluded the original translocation of 1,285 mottled ducks was sufficient to maintain current genetic diversity. We identified 7 hybrids, including an F1 and 3 late-staged (i.e., F2–F3 backcrosses) mottled duck × black duck hybrids, 1 F2-mottled duck backcrossed with a wild mallard, and 2 F3-mottled ducks introgressed with game-farm mallard. We estimated a 15% hybridization rate in our mottled duck dataset; however, the general lack of F1 and intermediate hybrids were inconsistent with scenarios of high hybridization rates or presence of a hybrid swarm. Instead, our results suggested a scenario of infrequent interspecific hybridization between South Carolina's mottled ducks and congeners. We concluded that South Carolina's mottled duck population is sufficiently large now to absorb current hybridization rates because 85% of sampled mottled ducks were pure. These results demonstrate the importance in managing and maintaining large parental populations to counter hybridization. As such, future population management of mottled ducks in South Carolina will benefit from increased geographical and continued sampling to monitor hybridization rates with closely related congeners. We also suggest that any future translocations of mottled ducks to coastal South Carolina should originate from the Western Gulf Coast. © 2021 The Wildlife Society.

**KEY WORDS** *Anas*, conservation, ddRAD-seq, genetics, hybridization, mallard, mottled duck, translocation.

Wildlife conservationists have translocated species outside of their native range to decrease potential for species extinction, particularly those with small population sizes or range limitations (Mock et al. 2004, Reynolds and Klavitter 2006, Braun et al. 2011, Downey et al. 2017, Gille et al. 2019). Translocations of waterfowl and other wildlife have also been performed for human recreation such as hunting (Holevinski et al. 2006, Weng 2006). Assessing outcomes of

translocations requires understanding potential effects of possible founder effects and subsequent inbreeding, hybridization with native congeners, and other genetic-behavioral consequences. Among concerns regarding translocations or reintroduction is the possibility of interbreeding with local congeners (Allendorf et al. 2001, Lavretsky et al. 2019, McFarlane and Pemberton 2019, Caniglia et al. 2020), with extreme cases resulting in hybrid swarms and failed conservation efforts (Wells et al. 2019). Thus, determining baseline genomics assists in whether translocation events successfully resulted in a breeding population and are important for future management actions to maintain the populations.

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Given that hybridization is prevalent in birds, especially in ducks (e.g., Anatinae; Ottenburghs et al. 2015), establishing populations near sexually non-isolated congeners may result in genetic swamping (Wells et al. 2019). Thus, determining extent and biological outcome from hybridization events is essential for sustaining species (Lavretsky et al. 2019). Specifically, hybridization occurs when pure parental taxa interbreed and produce a potentially viable  $F_1$  hybrid. For gene flow to occur, the  $F_1$  hybrid backcrosses with 1 or both parental taxa, effectively moving genes between taxa. Thus, whereas the biological outcome of this scenario is simply lost breeding potential within the subsequent breeding period of the hybridization event, gene flow has more cross-generational effects, with the potential of species or population loss if adequately severe. In general, genetic swamping is often proportional to population size (Mills and Allendorf 1996, Allendorf et al. 2001, Lavretsky et al. 2019); thus, a sufficiently large parental gene pool is required for hybrids to backcross continuously with to decrease potential negative effects of gene flow (Lavretsky et al. 2016).

The South Carolina Department of Natural Resources and cooperators translocated mottled ducks (*Anas fulvigula*) to South Carolina, USA, from 1975–1983 to expand the range of the species into coastal wetlands of the state that were similar to wetlands in the Gulf states and to establish hunting opportunities of the species established (LaHart and Cornwell 1970, Kneece 2016). Mottled ducks ( $n=1,285$ ) were moved to the state's Santee and Ashepoo, Combahee, and Edisto (ACE) basins from Florida ( $n=26$ ), Louisiana ( $n=1,117$ ), and Texas, USA ( $n=142$ ; Weng 2006, Shipes 2014, Kneece 2016). In 2008, there was an estimated population of 23,000 mottled ducks in South Carolina (Kneece 2016). Currently, mottled ducks generally occur only in coastal counties of South Carolina (Kneece 2016); however, the South Carolina population has expanded to Georgia (Baldassarre 2014, Pollander et al. 2019).

Stocks of mottled ducks used in these translocations were composed of 2 subspecies that are distinct in genetics, phenotype, and ecology (Bielefeld et al. 2010, 2016; Baldassarre 2014; Peters et al. 2016). In short, Western Gulf Coast mottled ducks (*A. f. maculatus*) are indigenous to coastal marshes surrounding the Gulf of Mexico from Mexico and north and east through Texas, Louisiana, Mississippi, and Alabama, USA. The subspecies can be found in shallow, fresh to saline marshes to inshore rice fields and wetlands within cattle pastures (Stutzenbaker 1988, Bielefeld et al. 2010). In contrast, the Florida mottled duck (*A. f. fulvigula*) is endemic to Florida's interior wetlands where the species uses brackish marshes, freshwater prairie and pasture ponds, and urban ponds, ditches, and storm-water impoundments (LaHart and Cornwell 1970, Bielefeld et al. 2010). Despite both subspecies occupying similar land cover types, hybridization has not been demonstrated between the 2 subspecies, perhaps because of their non-migratory nature (Peters et al. 2016). Although previous research suggested the genetic source for mottled

ducks in South Carolina originated mostly from the Western Gulf Coast region, these studies either were limited in sample size (Peters et al. 2016) or molecular markers (Williams et al. 2005, Weng 2006) and lacking in  $\geq 1$  possible taxa to address the question of geographical source. Moreover, translocations often suffer from high rates of genetic drift and inbreeding due to insufficient genetic variation as a result of few individuals within a founding population (Hedrick and Kalinowski 2000, Jamieson 2011). Thus, the extent that founding Western Gulf Coast and Florida mottled ducks contributed to the genetics of mottled ducks established in South Carolina, and whether these introduced populations have suffered from founder events (e.g., lacking genetic diversity and high levels of inbreeding) remained unknown.

Our objective was to understand the population structure and genetic diversity of South Carolina's extant mottled duck population. Given that 98% of all mottled ducks translocated to South Carolina originated from the Western Gulf Coast region, we predicted that extant South Carolina mottled ducks would assign genetically as Western Gulf Coast mottled ducks. Next, we estimated overall rates of possible hybridization between introduced mottled ducks in South Carolina and congeners found in the area. Previously, Williams et al. (2005) attributed lack of genetic diagnoses between mallards (*Anas platyrhynchos*) and mottled ducks from South Carolina to extensive gene flow between these congeners. If gene flow with mallards (wild or domestic) had amalgamated the genome of mottled ducks in South Carolina, then we would expect to find no pure mottled ducks and rather a hybrid swarm among sampled birds. Finally, we seized the opportunity to establish the source of game-farm mallards being released in South Carolina hunting preserves. We predicted that these game-farm mallards are likely of the same Eurasian origins as established for game-farm mallards sampled in hunting preserves elsewhere in North America (Lavretsky et al. 2020). Determination of feral  $\times$  wild mottled duck hybrids will help establish whether locally released game-farm mallards pose a genetic threat to wild mallard-like ducks in South Carolina and along the south Atlantic coast.

## STUDY AREA

The study spanned the 2016–2017 (19–26 Nov, 10 Dec–29 Jan) and 2017–2018 (18–25 Nov, 9 Dec–10 Jan) waterfowl hunting seasons. Weather during the 2016–2017 hunting season was characterized by above average temperatures and precipitation ranging from below average in November (temp = 13°C, precipitation = 0.5 cm) to above average in January (temp = 11°C, precipitation = 5 cm). Weather during the 2017–2018 hunting season was characterized by a range of temperatures. November was average (temp = 13°C, precipitation = 4 cm), December was above average (temp = 9°C, precipitation = 9 cm), and January was below average (temp = 5°C, precipitation = 11 cm).

We collected wing or breast muscle samples from individual ducks from several sources in South Carolina: 40 hunter-taken mottled ducks on state- or privately owned

lands in coastal South Carolina, 38 game-farm mallards from 2 private hunting clubs, and 3 black ducks (*Anas rubripes*) and 3 mallards opportunistically collected (Table S1, available in Supporting Information). South Carolina Department of Natural Resources public lands included Bear Island in the ACE Basin (1,400 km<sup>2</sup>; 32.612302 latitude, -80.443065 longitude) and Santee Coastal Reserve Wildlife (Waterfowl) Management Areas in the Santee River Delta Basin (300 km<sup>2</sup>; 33.126026 latitude, -79.310721 longitude; Table S1).

## METHODS

### Sampling Effort and Sequencing

We salvaged 84 samples from hunter-shot birds under a United States Fish and Wildlife Service Standard Conditions Migratory Bird Scientific Collecting Permit (MB11579C). We extracted genomic DNA from tissues using a DNeasy Blood and Tissue kit following the manufacturer's protocol (Qiagen, Valencia, CA, USA). We ensured DNA quality based on the presence of high molecular weight bands visualized using gel electrophoresis with a 1% agarose gel, and quantified DNA concentration using a Qubit 3 Fluorometer (Invitrogen, Carlsbad, CA) to ensure a minimum concentration of 20 ng/ $\mu$ L. We followed procedures presented by Lavretsky et al. (2015) to create multiplexed double digest restriction-site associated DNA sequencing (ddRAD-seq) fragment libraries. In short, we enzymatically fragmented genomic DNA using SbfI and EcoRI restriction enzymes (New England Biolabs, Ipswich, MA, USA), and ligated Illumina TruSeq (Illumina, San Diego, CA) compatible barcodes that permitted future de-multiplexing. We pooled libraries in equimolar concentrations, and completed 150 base pair, single-end sequencing on an Illumina HiSeq. 4000 at the University of Oregon's Genomics and Cell Characterization Core Facility. We deposited Illumina reads in the National Center for Biotechnology Information's Sequence Read Archive (SRA; <http://www.ncbi.nlm.nih.gov/sra>; BioProject PRJNA745366 accession numbers SAMN20164881–SAMN20164964).

We de-multiplexed raw Illumina reads using the computational pipeline described by DaCosta and Sorenson (2014) and following steps outlined in Lavretsky et al. (2015). Prior to genotyping, we filtered all samples sequenced for 150 base-pair fragments to the first 100 base pairs to be comparable to previously published ddRAD-seq data sequenced using 100 base-pair chemistry (Peters et al. 2016). In addition to mottled ducks, American black ducks (i.e., black duck), wild mallards, feral mallards (i.e., mallards of domestic genetic origin), and any combination of hybrids among these are possible in South Carolina. Thus, we obtained previously published ddRAD raw sequence data generated using the same protocols as described above for mallards (wild mallards [Lavretsky et al. 2019], game-farm mallards [Lavretsky et al. 2020]), black ducks (Lavretsky et al. 2019), Western Gulf Coast mottled ducks (Peters et al. 2016), and Florida mottled ducks (Peters et al. 2016). We limited samples for black ducks and mallards to pure

representatives and excluded putative hybrids and samples recovered with any evidence of game-farm mallard nuclear introgression (Lavretsky et al. 2019). We combined our samples with reference samples and genotyped these following the DaCosta and Sorenson (2014) software pipeline. In short, the pipeline clusters filtered reads into putative loci based on sequence similarity and genomic position as determined by BLASTN version 2 (Altschul et al. 1990), to the reference mallard sequence (Huang et al. 2013), aligns reads within each putative locus, and infers haplotypes for individual samples at each locus. We were able to separate ddRAD autosomal versus Z-sex chromosome-linked loci by knowing chromosomal locations of our loci. All downstream analyses were based on ddRAD autosomal loci only. We further limited sequencing error by requiring a minimum sequencing depth of 5 reads to score an allele; otherwise, we scored the allele as missing. We retained loci with <20% missing genotypes for downstream analyses, and generated final output files (e.g., ADMIXTURE, fineRADstructure) with custom Python scripts (Lavretsky et al. 2016).

### Population Structure

To evaluate nuclear population structure, we used autosomal ddRAD-seq bi-allelic single nucleotide polymorphisms (SNPs) only. Prior to analyses, we used PLINK version 0.70 (Purcell et al. 2007) to ensure that singletons (i.e., minimum allele frequency [maf] = 0.004) and any SNP missing  $\geq 20\%$  of data across samples were excluded in each dataset. Additionally, we identified independent SNPs by conducting pair-wise linkage disequilibrium (LD) tests across ddRAD-seq autosomal SNPs (--indep-pairwise 2 1 0.5) in which 1 of 2 linked SNPs are randomly excluded if we obtained an LD correlation factor ( $r^2$ ) > 0.5. We conducted all analyses without *a priori* information on population or species identity.

First, we used the `dudi.pca` function in the R package Adegenet (Jombart 2008) to perform a principal component analysis (PCA). Next, we used ADMIXTURE version 1.3 (Alexander et al. 2009, Alexander and Lange 2011) to attain maximum likelihood estimates of population assignments for each individual, with datasets formatted for the ADMIXTURE analyses using PLINK version 0.70 (Purcell et al. 2007), and following steps outlined in Alexander et al. (2012). We ran each ADMIXTURE analysis with a 10-fold cross validation, and with a quasi-Newton algorithm employed to accelerate convergence (Zhou et al. 2011). Each analysis used a block relaxation algorithm for point estimation and terminated once the change in the log-likelihood of the point estimations increased by <0.0001. We ran ADMIXTURE for  $K$  populations of 1 through 5, and with 100 iterations per each value of  $K$ . The optimum  $K$  was based on the average of cross validation errors across the iterations per  $K$  value; however, we examined additional values of  $K$  to test for further structural resolution across analyses. We used the R package PopHelper (Francis 2016) to convert ADMIXTURE outputs into CLUMPP input files at each  $K$  value, and determine the robustness of the

assignments of individuals to populations at each  $K$  value with the program CLUMPP version 1.1 (Jakobsson and Rosenberg 2007). In CLUMPP, we employed the Large Greedy algorithm and 1,000 random permutations. Final admixture proportions for each  $K$  value and per sample assignment probabilities (Q estimates; the log likelihood of group assignment) were based on CLUMPP analyses of all 100 replicates per  $K$  value.

In addition, we assessed patterns of co-ancestry using fineRADstructure (Malinsky et al. 2018), which includes the programs RADpainter version 0.1 and finestructure (Lawson et al. 2012). In short, fineRADstructure derives a matrix of co-ancestry coefficients based on the distribution of identical or nearest neighbor haplotypes among samples. Each individual's co-ancestry at each locus is equally divided among all other individuals with identical haplotypes, or in the case of a unique allele, all other individuals with the nearest neighbor haplotype. Thus, rare haplotypes defined by rare SNPs, which are on average of more recent origin (Kimura and Ohta 1973), contribute the most to the co-ancestry index, providing a measure that emphasizes recent co-ancestry. This analysis is also completed without *a priori* information on population or species identity. We completed a burn-in of 100,000 iterations, followed by 100,000 Markov chain Monte Carlo iterations, followed by tree building using default parameters. To visualize the results, we used the R scripts `fineradstructureplot.r` and `finestructurelibrary.r` (R Core Team., 2020).

Finally, we calculated composite pairwise estimates of relative divergence ( $\Phi_{ST}$ ) and nucleotide diversity across our sampled groups using a separate concatenated dataset of ddRAD autosomal loci or the mtDNA control region in the R package PopGenome (Pfeifer et al. 2014); we excluded insertions or deletions (indels) from analyses. We excluded potential hybrids or samples taxonomically misidentified (e.g., a mottled duck that was genetically a black duck) in analyses.

### Establishing Hybrid Indices and Hybrid Assignment

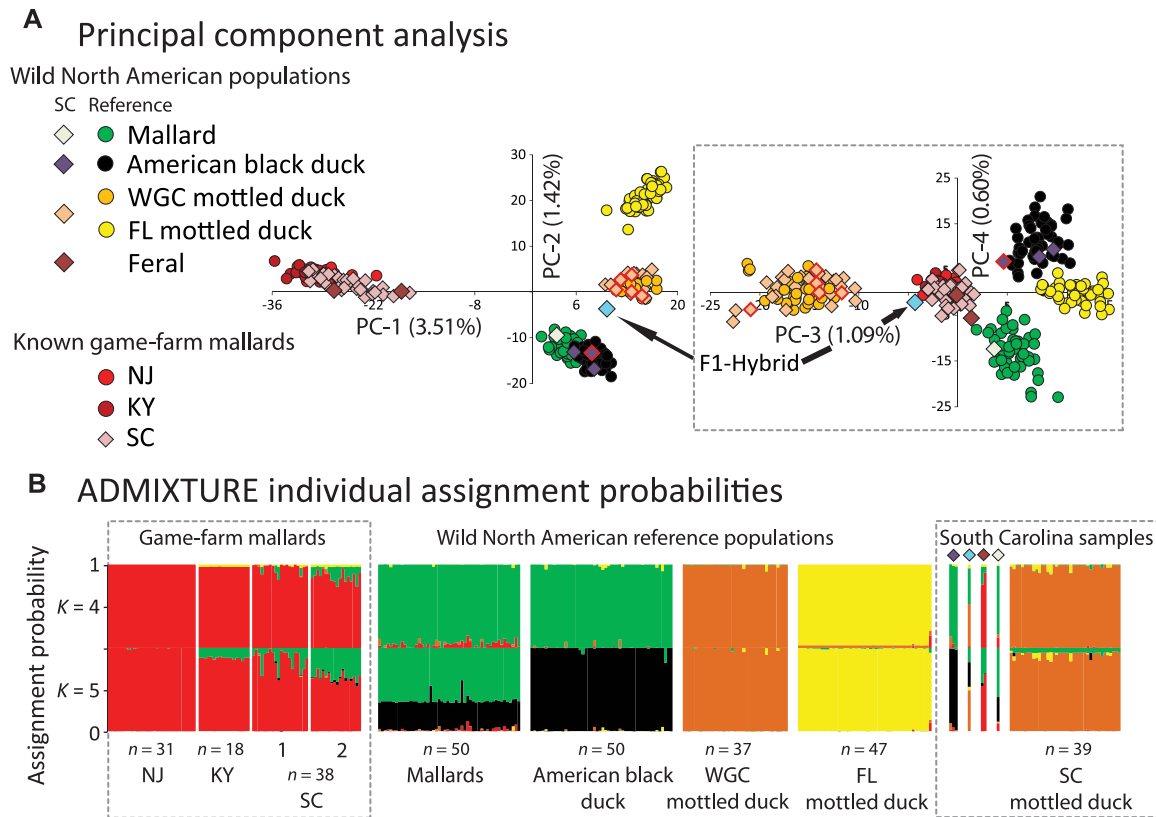
To assess the effectiveness of our molecular dataset in distinguishing between classes of generational hybrids and to more directly assign putative hybrids to those classes, we simulated expected assignment probabilities with our empirical data for first generation hybrids (F1) and 9 generations of backcrosses (F2–F10) based on methods outlined in Lavretsky et al. (2016). If hybridization was continuous and widespread, then we would expect to find many individuals with equivalent F1 assignment probabilities. If hybridization occurred but was infrequent, then we expected to find most hybrids to be late stage ( $>F2$ ). Alternatively, if past hybridization had been so extensive that no pure mottled ducks exist, then we expected to find all samples with assignment probabilities intermediate between the simulated expectations of F1–F10 generations (Lavretsky et al. 2019).

We used the same ddRAD-seq bi-allelic nuclear SNP set analyzed for population structure in simulations, providing us the expected and generational assignment limits given

our data. We conducted 2 separate breeding simulations between sampled mottled ducks in South Carolina and wild mallards and black ducks because these were the most likely congeners to interbreed with mottled ducks. We also used the wild mallard simulations as a proxy for any possible South Carolina mottled duck and game-farm mallard hybrids. In short, we first generated 10 F1 hybrids by randomly sampling an allele from the mottled ducks in South Carolina and either the wild mallard or black duck gene pool across bi-allelic SNP positions; we randomly sampled each position based on a probability proportional to the allelic frequency in each respective gene pool. We then backcrossed 5 hybrids to the parental gene pool for 9 generations. To limit potential biases in simulations, we reconstructed hybrid indices using only individuals with initial ADMIXTURE-based probabilities of  $\geq 95\%$  assignment to mottled ducks in South Carolina and either wild mallards or black ducks. General mottled duck ancestry among South Carolina samples was the summation of assignment to Florida and Western Gulf Coast mottled ducks (Fig. 1), allowing us to capture their genetic diversity within simulations. We ran 10 independent simulations, with data subsequently inputted into ADMIXTURE to estimate assignment probabilities for a  $K$  of 2. At each  $K$ , we ran 25 iterations per simulation for 250 ADMIXTURE outputs generated per  $K$ , which we then combined and converted in PopHelper (Francis 2016) into CLUMPP input files. We employed the Large Greedy algorithm and 1,000 random permutations with final admixture proportions for each  $K$  and per sample assignment probabilities based on CLUMPP analyses of all 250 replicates per  $K$ . Per generation expected assignment probabilities were based on the average of all 10 (F1) or each of the 5 (F2–F10) backcrosses, along with each lower and upper limit.

### Mitochondrial DNA

In addition to nuclear markers, we sequenced the mitochondrial DNA (mtDNA) control region for which there are 2 divergent mtDNA haplogroups: Old World (OW; Eurasian origin) A and New World (NW; North American origin) B (Ankney et al. 1986, Avise et al. 1990, Lavretsky et al. 2014a). We used primers L78 and H774 to polymerase chain reaction (PCR) amplify and sequence 625 base pairs of the mtDNA control region (Sorenson and Fleischer 1996, Sorenson et al. 1999) following Sanger Sequencing methods described in Lavretsky et al. (2014b). We sequenced the PCR products using the L78 primer on a 3130XL Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) at the University of Texas at El Paso, Border Biomedical Research Center's Genomic Analysis Core Facility. We aligned and edited sequences using Sequencher version 4.8 (Gene Codes, Ann Arbor, MI, USA). All sequences have been submitted to GenBank (accession numbers MZ594912–MZ594995). We were able to obtain mtDNA control region sequences for the same set of wild mallard, game-farm mallard, and black duck reference samples (Lavretsky et al. 2014a, b, 2019, 2020) as for ddRAD-seq but used another set of published samples for Western Gulf Coast (KJ791982–KJ792001



**Figure 1.** A) Principal components analysis (PCA) results for North American reference populations of sampled game-farm mallards from New Jersey, Kentucky, and South Carolina, USA, wild mallards, American black ducks, Western Gulf Coast (WGC) mottled ducks, Florida mottled ducks, and South Carolina samples, with plots comparing principal component (PC) 1 versus PC 2 and PC 3 versus PC 4 scores. B) ADMIXTURE assignment probabilities for  $K$  populations of 4 and 5 for the same set of samples as in the PCA. All putative hybrids are red bolded in the PCA, and sample sizes are denoted under each sampled species or population in the ADMIXTURE plot. For South Carolina, we sampled during the waterfowl hunting seasons of 2016–2017 and 2017–2018.

[Lavretsky et al. 2014a], KF608530–KF608534 [Lavretsky et al. 2014b]) and Florida (KJ791963–KJ791981 [Lavretsky et al. 2014a], KF608525–KF608529 [Lavretsky et al. 2014b]) mottled ducks. We inferred a mtDNA haplotype network using a median-joining algorithm in the program Network version 5.0 (Bandelt et al. 1999). We also used the output from the Network tree to determine which samples possessed Old World (A) versus New World (B) mitochondrial haplotypes.

## RESULTS

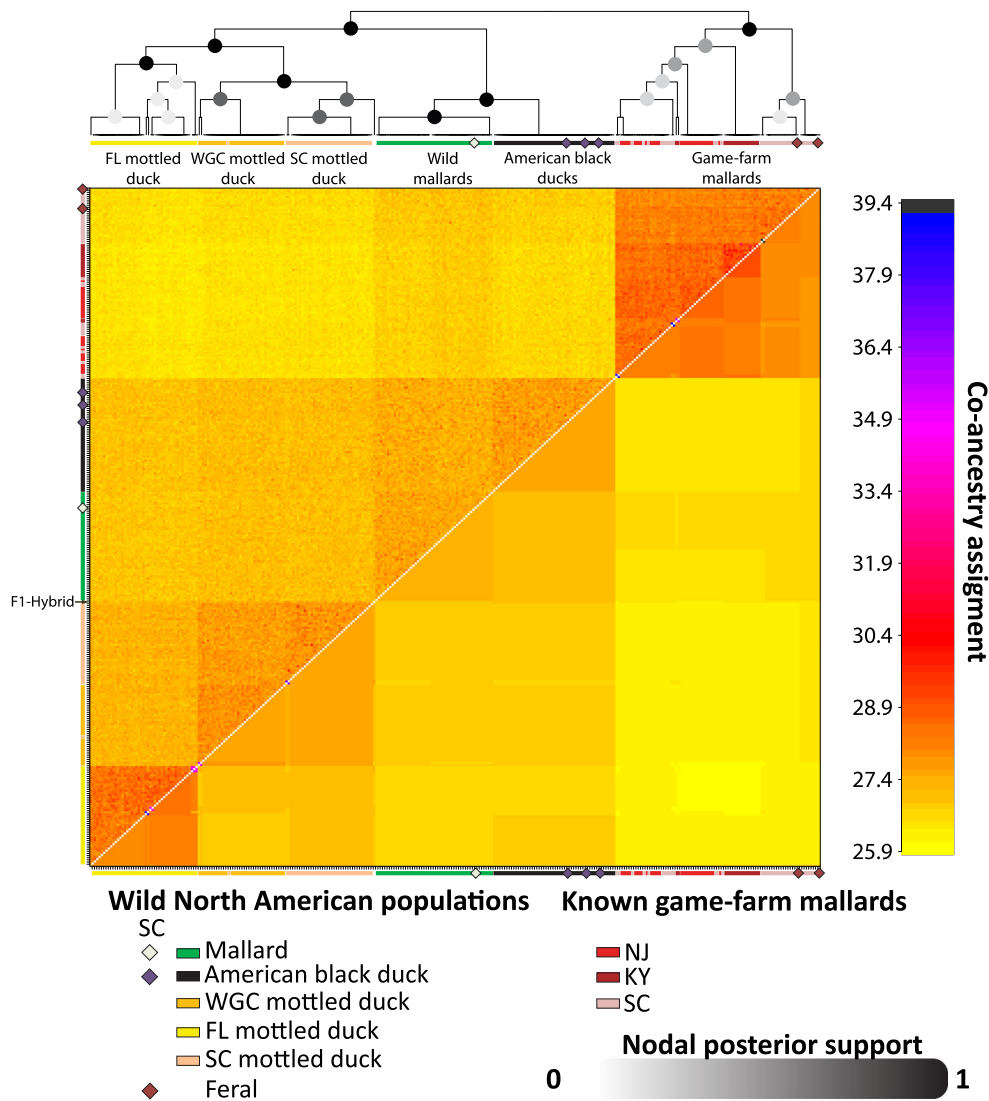
For our nuclear dataset, 2,354 ddRAD-seq autosomal loci (211,609 base pairs and 9,852 bi-allelic SNPs) met our sequencing coverage and missing data criteria. We obtained an average median depth of 110 sequences/locus, with a median sequencing depth range of 23–743 sequences across samples. We attained 625 overlapping base pairs of the mtDNA control region across samples.

### Population Structure

We based our population structure analyses on 8,574 ( $n=9,852$ ) independent bi-allelic SNPs. We detected concordance among PCA, ADMIXTURE, and fineRADstructure analyses (Figs. 1 and 2). First, plotting the first 2 components of the PCA differentiated among Florida and Western Gulf Coast

mottled ducks and game-farm mallards, but wild mallard and black duck samples were separated by plotting principal components 3 and 4 (Fig. 1A). Next, ADMIXTURE analysis concluded an optimum  $K$  population of 4 (Fig. S1, available in Supporting Information); however, we obtained resolution between wild mallards and black ducks by analyzing at a  $K$  population value of 5 (Fig. 1B). Moreover, our co-ancestry estimates in the fineRADstructure analysis revealed the same 5 major clusters, with significant nodal support for them in the dendrogram (Fig. 2).

Of the 40 putative mottled ducks that we sampled in South Carolina, 39 ducks had assignment probabilities of  $\geq 90\%$  to the reference Western Gulf Coast mottled duck genetic group (Fig. 1B) and clustering with Western Gulf Coast mottled ducks based on our PCA (Fig. 1A) and fineRADstructure co-ancestry matrix (Fig. 2). The ADMIXTURE analysis of the 39 samples revealed assignment probabilities of approximately 5–10% to Florida mottled ducks ( $n=6$ ) and black ducks ( $n=2$ ; Fig. 1B), with the latter determined to be late stage mottled duck  $\times$  black duck crosses. A single sample initially identified as mottled duck was determined to be an F1 mottled duck  $\times$  black duck hybrid. All game-farm mallards from South Carolina closely clustered and were assigned to the same genetic group as game-farm mallards from New Jersey and Kentucky.



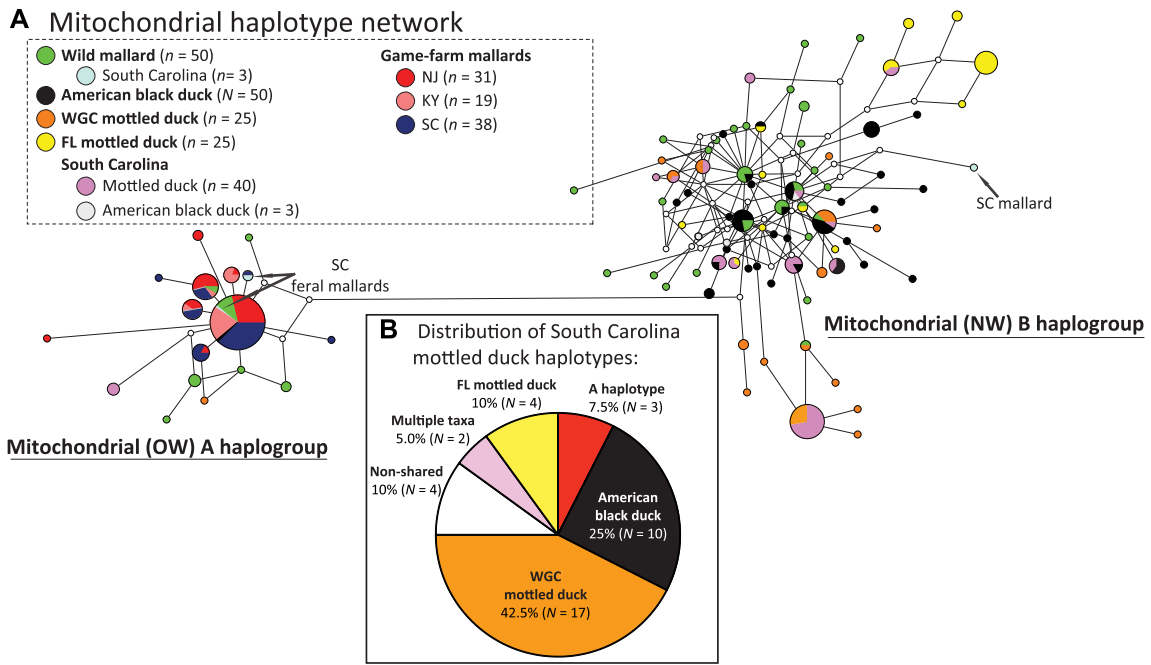
**Figure 2.** FineRADstructure individual (above diagonal) and average (below diagonal) co-ancestry coefficient matrix for sampled North American reference populations of game-farm mallards from New Jersey, Kentucky, and South Carolina, USA, wild mallards, American black ducks, Western Gulf Coast (WGC) mottled ducks, Florida mottled ducks, and South Carolina samples. Samples are color coded by reference species or population. The level of recent co-ancestry is color coded from low (yellow) to high (blue). Additionally, posterior supports across dendrogram branches are provided. For South Carolina, we sampled during the waterfowl hunting seasons of 2016–2017 and 2017–2018.

Game-farm mallards from 1 of the 2 sampled hunting preserves in South Carolina had greater assignment probability (i.e., >10%; Fig. 1B) and co-ancestry (Fig. 2) with wild mallards compared to remaining game-farm mallards. For 3 hunter-shot mallards from South Carolina, 1 was determined to be a wild North American mallard, whereas the other 2 were feral game-farm mallards (Figs. 1 and 2). All 3 black ducks from South Carolina clustered with reference black ducks (Figs. 1 and 2).

We recovered known OW A and NW B haplogroups in our mtDNA haplotype network (Fig. 3A). First, all game-farm mallards from South Carolina carried 7 OW haplotypes, including 4 haplotypes shared with other game-farm mallards from New Jersey or Kentucky and 3 haplotypes that were specific to South Carolina. Both feral mallards carried OW A mtDNA haplotypes, with 1 being the dominant haplotype within the OW A haplogroup, and

the other shared with a game-farm mallard from South Carolina. All 3 black ducks collected in South Carolina were within the NW B haplogroup; 2 samples had unshared haplotypes, and the other a shared haplotype with the other representative black ducks. Among the remaining 40 samples, 92.5% ( $n=37$ ) and 7.5% ( $n=3$ ) carried NW B and OW A haplotypes, respectively (Fig. 3). Only 3 mtDNA haplotypes ( $n=4$  samples from SC) were not shared with any other taxa (Fig. 3B). Of remaining haplotypes, 42.5%, 25.0%, and 10.0% exclusively were shared with Western Gulf Coast mottled ducks, black ducks, and Florida mottled ducks, respectively (Fig. 3B).

We estimated relative genetic differentiation ( $\Phi_{ST}$ ) using South Carolina samples that genetically were identified as mottled duck or game-farm mallard only (i.e., no hybrids), and treated game-farm mallards from New Jersey and Kentucky as a single game-farm mallard lineage. South

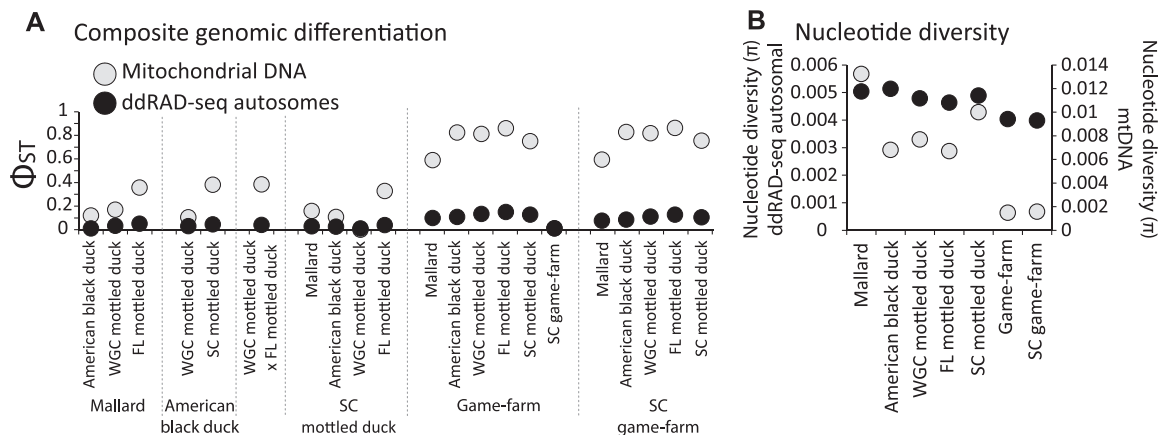


**Figure 3.** A) A haplotype network reconstructed from 625 base pairs of the mitochondrial control region for sampled North American reference populations of game-farm mallards from New Jersey, Kentucky, and South Carolina, USA, wild mallards, American black ducks, Western Gulf Coast (WGC) mottled ducks, Florida mottled ducks, and South Carolina samples. Sampled groups are color coded or indicated on the network, including respective sample sizes. Circles denote different haplotypes with circle size proportionate to the number of samples represented within the haplotype. The length of the connecting line between haplotypes is proportionate to the number of mutations separating 2 haplotypes. B) The proportion (and total) of mottled duck samples from South Carolina that are exclusively shared with game-farm mallards (i.e., A haplotypes), American black ducks, WGC mottled ducks, or Florida mottled ducks, are exclusive to mottled ducks in South Carolina (non-shared), or are shared with multiple taxa; no haplotypes were exclusively shared with wild mallards. For South Carolina, we sampled during the waterfowl hunting seasons of 2016–2017 and 2017–2018.

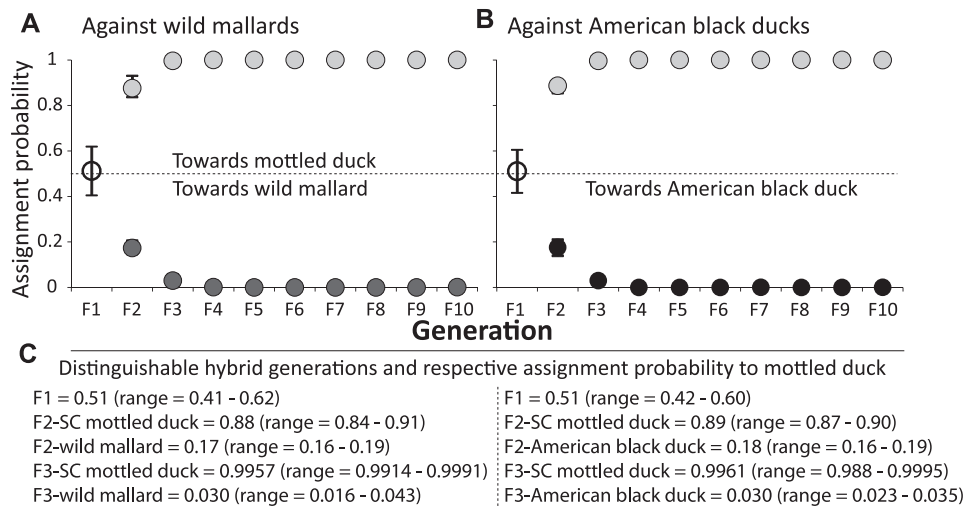
Carolina mottled ducks were not genetically distinguishable from Western Gulf Coast mottled ducks at mtDNA ( $\Phi_{ST} = -0.0019$ ) and nuclear (composite  $\Phi_{ST} = 0.010$ ; Fig. 4A) markers. Game-farm mallards from South Carolina were similar to reference game-farm mallards at mtDNA ( $\Phi_{ST} = 0.011$ ) and nuclear ddRAD-seq loci (composite  $\Phi_{ST} = 0.010$ ) and equally differentiated from all other sampled wild reference populations and South Carolina mottled ducks as the reference set of game-farm mallards (Fig. 4A).

### Hybrid Indices and Hybrid Assignment

Simulated assignment probabilities for South Carolina mottled ducks and either wild mallards (Fig. 5A) or black ducks (Fig. 5B) distinguished between F1 hybrids and F2 and F3 backcrosses. We were able to identify the parental population for all  $\geq F4$  generational backcrosses (Table S2, available in Supporting Information). Comparing empirical assignment probabilities from our ADMIXTURE analysis under a  $K$  population of 5 to the distinguishable hybrid



**Figure 4.** A) Pairwise composite relative genetic differentiation ( $\Phi_{ST}$ ) estimates and B) overall nucleotide diversity across sampled North American reference populations of game-farm mallards, wild mallards, American black ducks, Western Gulf Coast (WGC) mottled ducks, Florida mottled ducks, and South Carolina samples and calculated from double digest restriction-site associated DNA sequencing (ddRAD-seq) autosomal or the mitochondrial DNA (mtDNA) control region. We treated game-farm mallards from New Jersey and Kentucky as a single game-farm mallard lineage. For South Carolina, sampling was done during the waterfowl hunting seasons of 2016–2017 and 2017–2018.



**Figure 5.** Simulated expected assignment probabilities based on double digest restriction-site associated DNA sequencing autosomal bi-allelic single nucleotide polymorphisms for 10 hybrid classes determined between mottled ducks in South Carolina, USA, and either A) wild mallards or B) American black ducks. C) Estimated average and range of assignment probability to the mottled duck genetic cluster in South Carolina for diagnosable hybrid classes, which are denoted as generation class (F1–F3) and to which they were backcrossed with South Carolina mottled duck, wild mallard, or American black duck. The dotted line denotes 50:50 assignment.

categories for each of our simulated datasets (Fig. 5C) identified 7 of our South Carolina samples to specific hybrid or intermediate classes. Samples with interspecific assignments intermediate to those recovered in simulated F1–F3 generations represented variation in backcrosses (e.g., hybrid  $\times$  hybrid crosses, parental hybridization switching between generations; Lavretsky et al. 2019). Moreover, with several of South Carolina's sampled mottled ducks showing small assignment probabilities (~5–10%) to the Florida mottled duck group (Fig. 1B), which we considered to be part of this populations ancestry and not distinguished in our simulations, a sample's mottled duck ancestry was the sum of assignment to both Florida and Western Gulf Coast mottled duck genetic clusters.

For the putative hybrids, 1 of the 3 black ducks had approximately 8% assignment probability to the mottled duck genetic cluster that was intermediate to the expected assignment probabilities of simulated F2- and F3-black duck backcrossed with mottled duck (Fig. 5C). A single sample that was intermediate across population structure analyses (Figs. 2 and 3) was equivalent to a simulated F1 hybrid (Fig. 5C). We concluded it was an F1 mottled duck  $\times$  black duck hybrid given the sample possessed equivalent co-ancestry between these 2 populations and not with mallards (Fig. 2). Among the 5 South Carolina samples that genetically clustered among mottled ducks (Fig. 3A), 2 possessed approximately 9% (i.e., F2-mottled duck backcrossed with black duck) and 4% (i.e., F3-mottled duck backcrossed with black duck) black duck, another possessed approximately 5% wild mallard (i.e., F2-mottled duck backcrossed with mallard), and 2 possessed approximately 1–2% game-farm mallard (i.e., F3-mottled duck backcrossed with game-farm mallard). One of the 2 samples considered to be an F3-mottled duck backcrossed with game-farm mallard also possessed an OW A mtDNA haplotype, which

substantiates interbreeding between mottled ducks and game-farm mallards, and that the source had to be a female game-farm mallard in this case. We determined the remaining 34 (85%) of mottled duck samples from South Carolina to be genetically pure (Table S1).

### Molecular Diversity

Molecular diversity at nuclear and mtDNA sites for South Carolina mottled ducks was similar to those calculated for other wild reference populations of mallards, black ducks, and Western Gulf Coast and Florida mottled ducks (Fig. 4B). Mottled ducks in South Carolina had on average 1.4 times greater mtDNA diversity but near similar nuclear diversity when compared to the reference Florida and Western Gulf Coast mottled ducks. Calculated genetic diversity for game-farm mallards from South Carolina was similar to those reported in other game-farm mallards in North America (Fig. 4B). In general, sampled wild populations of mallards ( $\pi_{\text{mtDNA}} = 0.013$ ,  $\pi_{\text{nuclear}} = 0.0050$ ), black ducks ( $\pi_{\text{mtDNA}} = 0.0068$ ,  $\pi_{\text{nuclear}} = 0.00051$ ), Western Gulf Coast mottled ducks ( $\pi_{\text{mtDNA}} = 0.0077$ ,  $\pi_{\text{nuclear}} = 0.00048$ ), and Florida mottled ducks ( $\pi_{\text{mtDNA}} = 0.0067$ ,  $\pi_{\text{nuclear}} = 0.00046$ ) each have on average 6- and 1.2-fold greater diversity at mtDNA and nuclear genomes, respectively, than game-farm mallards ( $\pi_{\text{mtDNA}} = 0.0015$ ,  $\pi_{\text{nuclear}} = 0.00040$ ; Fig. 4B).

## DISCUSSION

We provided a genetic assessment of mottled ducks translocated to South Carolina wherein now a breeding population exists. Although mottled ducks from Florida and the Western Gulf Coast region were used in introduction efforts between 1975 and 1983, the genetic signature in mottled ducks sampled in our study was overwhelmingly from the latter region, where approximately 98% of the translocated birds originated (Weng 2006, Shipes 2014,



Kneece 2016). Additionally, PCA clustering, calculated assignment probabilities, and co-ancestry estimates all evidenced that mottled ducks largely possessed the same genetic signature as the Western Gulf Coast subspecies. Moreover, our mtDNA haplotype network revealed a high degree of haplotype sharing with Western Gulf Coast mottled ducks. This result further corroborated the lack of measurable genetic differentiation between the South Carolina and Western Gulf Coast populations at both nuclear and mtDNA markers. Therefore, we submit the current South Carolina population of mottled ducks legitimately can be classified taxonomically as the Western Gulf Coast subspecies.

Representing approximately 2% of the founding population, Florida mottled ducks introduced to South Carolina may have been genetically swamped, succeeded by Western Gulf Coast mottled ducks, exist in areas of South Carolina not sampled during our study, or a combination of these or other effects. We contend that the complete nuclear-based co-ancestry sharing with Western Gulf Coast mottled ducks, despite the 2 Florida mottled duck mtDNA haplotypes, suggests that Florida mottled ducks in the sampled areas likely were genetically swamped and replaced by the overwhelming number of Western Gulf Coast mottled ducks. Whereas mtDNA persists in lineages longer than nuclear DNA (Hurst and Jiggins 2005, Peters et al. 2014), our simulations suggest that the backcrossing of as few as 3 or 4 generations into the same parental population can cause a complete genetic replacement at nuclear DNA (Lavretsky et al. 2016, 2019). The amount of Florida mottled duck genetic assignment among some South Carolina mottled ducks was similar to levels found in several reference Western Gulf Coast mottled ducks, suggesting these likely represent ancestry rather than hybridization. Additionally, the success of Western Gulf Coast over Florida mottled ducks in these introductions may be in part due to differences in environmental adaptations between the subspecies. Specifically, the Gulf of Mexico and South Carolina coastal wetlands are similar environments (Stutzenbaker 1988, Bielefeld et al. 2010), and thus Western Gulf Coast mottled ducks may have adapted to these systems better than Florida mottled ducks from inland fresh water wetlands of that state. Future research will benefit from additional sampling to determine whether Florida mottled ducks exist or have been amalgamated now into the genome of South Carolina mottled ducks.

Game-farm mallards from 2 hunting clubs in South Carolina revealed low levels of genetic differentiation at nuclear and mtDNA sites compared to other game-farm mallards sampled in North America (Lavretsky et al. 2019, 2020). All South Carolina game-farm mallards were found within the Eurasian-derived OW A haplogroup, with individuals sharing all major haplotypes recovered in other game-farm mallards. Nuclear variation corroborated mtDNA, with PCA clustering and co-ancestry estimates placing game-farm mallards from South Carolina with previously sampled game-farm mallards. We observed that all game-farm mallards showed slightly elevated co-ancestry

with wild mallards, which is expected because wild Eurasian mallards were original ancestors of domestic Nearctic mallards (Kiple 2001, Huang et al. 2013). Possessing OW A haplotypes and the same nuclear genomes further supported that game-farm mallards in North America are descendants of Eurasian domestic stock (Lavretsky et al. 2020) that were imported and since perpetuated across North America by waterfowl hunting preserves and clubs (Heusmann 1974, Soutiere 1986, Heusmann 1991). Samples from 1 of the 2 South Carolina clubs showed an increased degree of genetic assignment in ADMIXTURE analyses to wild mallards as compared to other game-farm mallards. This finding is consistent with anecdotal reports that some game-farm mallard breeders in South Carolina have captured and introduced wild mallards from the Mississippi flyway into their flocks to boost their genetic diversity in the recent past (R. K. Kaminski, Clemson University, personal communication).

Of the 3 hunter-harvested mallards opportunistically collected in South Carolina where mallards were not released, 2 had the same nuclear assignment, clustering, co-ancestry, and mtDNA haplotypes as game-farm mallards from South Carolina. Thus, we concluded these birds represented feral mallards and provide evidence that game-farm mallards survive and disperse beyond their release site. More importantly nuclear DNA identified 2 putative F3-mottled ducks backcrossed with game-farm mallard, with 1 confirmed by the presence of an OW A mtDNA haplotype. Despite being pure mottled ducks via nuclear DNA, another 2 samples possessed OW A mtDNA haplotypes, suggesting an older ( $\geq F4$ ) introgression event in their respective lineage's history. Though the true number of game-farm mallards released annually in South Carolina is not known, anecdotal reports suggest that 30,000–40,000 game-farm mallards are released annually. Despite a relatively low rate of hybridization (i.e., ~10%;  $n = 4$ ) between mottled ducks and game-farm mallards in South Carolina, our evidence suggests that some proportion of these game-farm mallards indeed survive outside their release site and interbreed with local congeners (Lavretsky et al. 2019, 2020). Being under artificial selection for desired domestic traits (e.g., breeding propensity), all domestic mallards differ in fertility, overall morphology, and biology from their wild counterparts (Miller 1977, Paulke and Haase 1978, Söderquist et al. 2013, Svobodová et al. 2020). The movement of their genetic variation and associated maladaptive traits into wild populations may reduce survival and fecundity of wild mottled ducks (Söderquist et al. 2014, McFarlane and Pemberton 2019, Svobodová et al. 2020, Söderquist et al. 2021). Feral mallards now pose a genetic threat to global populations of wild mallard and mallard-like taxa, with confirmed feral  $\times$  wild hybrid swarms in Eurasia (Söderquist et al. 2014, 2017), mainland North America (Lavretsky et al. 2019, 2020), and Hawaii (Wells et al. 2019), all of which show declining populations. Future research will benefit from continued genetic monitoring to determine true rates and potential consequences from game-farm mallard  $\times$  mottled duck interbreeding across

South Carolina and the dispersion of these elsewhere throughout North America.

### **Genetics of Translocated Wildlife and Importance of Population Size**

Prevalent interspecific hybridization is a reality for many translocation management efforts involving relatively few individuals; thus, genetic monitoring of introductions and reintroductions is essential to assess their success. Until recently, most molecular-based studies suffered in the number of genetic markers and samples to assign individuals to different hybrid groups (Caniglia et al. 2020, Leipold et al. 2020). Potentially biased conclusions resulting from too few molecular markers is exemplified by the contradicting result of diagnoses that we present compared to Williams et al. (2005); they concluded that mallards and mottled ducks from South Carolina were not genetically distinguishable. Decision making during translocation of mottled ducks to South Carolina would have been more efficient had current knowledge regarding the genetic distinctiveness of the 2 mottled duck subspecies been available (Peters et al. 2016). Additionally, determining success of translocation efforts requires reference populations. References provide comparative baseline molecular diversity to determine whether the translocated population experienced a founder effect. Unlike other introductions or translocations where bottlenecks may occur (Mock et al. 2004, Reynolds et al. 2015, Parra et al. 2018), we found no evidence of genetic drift (i.e., founder effects) or inbreeding (see co-ancestry estimates). Mottled ducks in South Carolina had on average 1.4 times greater mtDNA diversity and near similar nuclear diversity to their source populations, which we attribute to them possessing an array of haplotypes derived from their originating populations (i.e., Florida and Western Gulf Coast mottled ducks) and potentially through previous introgression with black ducks, wild mallards, or domestic mallards after translocation to South Carolina. Our genetic assessment permitted us to conclude the translocation of 1,285 mottled ducks to South Carolina was sufficiently large and diverse to avoid issues of inbreeding. Moreover, comparing our South Carolina samples to large reference sample sets (Nazareno et al. 2017, Leipold et al. 2020) enabled us to determine the relative genetic contribution from the original parental populations of mottled ducks and establish hybrid identities.

Finally, simulating expected genetic assignment probabilities from breeding between South Carolina mottled ducks and either wild mallards or black ducks established the capacity of our molecular marker set to differentiate between different hybrid generations. When compared to simulated hybrid classes, all but 1 of the 7 putative hybrids were characterized as F2- or F3- generational backcross (Table S1). The lack of F1 and intermediate hybrids among phenotypic mottled ducks is inconsistent with scenarios of high hybridization rates or the presence of a hybrid swarm, respectively, and rather suggests a scenario of infrequent hybridization for South Carolina's mottled duck population. Of 7 hybrids, 4 were identified to be with black ducks,

whereas 1 and 2 were with wild and game-farm mallards, respectively. Additional sampling will be required to determine whether hybridization between mottled and black ducks is truly greater than with mallards. Regardless, these results are inconsistent with earlier claims that hybridization between mallards and mottled ducks in South Carolina has resulted in a hybrid swarm (Williams et al. 2005). We hypothesize that differences in habitat use (i.e., mottled ducks in coastal habitats vs. wild and game-farm mallards largely in inland habitats; Mastro 2019), and potentially seasonally early pair formation in mottled ducks (Paulus 1988) may be important isolating mechanisms maintaining low hybridization rates with wild and feral mallards and black ducks. Although we conclude that South Carolina's mottled duck population appears to be sufficiently large to absorb current hybridization rates, research is needed to improve precision of mottled duck nest success estimates because existing estimates may be below levels to sustain the South Carolina population (i.e., <15%; Cowardin et al. 1985, Kneece 2016), which can result in future genetic swamping (Wells et al. 2019).

### **MANAGEMENT IMPLICATIONS**

Our study outlined a protocol for evaluating the genetic structure and diversity of translocated mottled ducks and other waterfowl or avian populations from which tissue or blood can be harvested. In addition to the thousands of molecular markers that are now reliably and cost-effectively attained with methods like ddRAD-seq and that distinguish between closely related taxa and their hybrids, we demonstrated that reference samples were critical to assess potential multi-species hybrids. We concluded that current hybridization rates (~15%) of mottled ducks appear to be insufficient to cause complete genetic swamping; however, this claim could change if the mottled duck population in South Carolina decreases or hybridization rates increase. Thus, future surveys will benefit from increased sampling of areas where mottled ducks co-occur with released, feral, and migratory populations of congeners to understand and monitor the extent to which hybridization poses a threat through time. Moreover, we recommend that recruitment rates of the South Carolina mottled ducks should be estimated to determine the population's trajectory, that future translocation efforts to South Carolina should use Western Gulf Coast mottled ducks only, and that the population should be sufficiently large (e.g., >1,200) to limit any founder effects.

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