The Journal of Wildlife Management 1–13; 2021; DOI: 10.1002/jwmg.22085

Research Article

Mallard–Black Duck Hybridization and Population Genetic Structure in North Carolina

DANIEL M. LAWSON,[1] *University of Delaware, 531 South College Avenue, Newark, DE 19716, USA*
CHRISTOPHER K. WILLIAMS, *University of Delaware, 531 South College Avenue, Newark, DE 19716, USA*
PHILIP LAVRETSKY, *University of Texas at El Paso, 500 W University Avenue, El Paso, TX 79968, USA*
DOUGLAS L. HOWELL, *North Carolina Wildlife Resources Commission, 132 Marine Drive, Edenton, NC 27699, USA*
JOSEPH C. FULLER, *North Carolina Wildlife Resources Commission, 132 Marine Drive, Edenton, NC 27699, USA*

ABSTRACT North Carolina, USA, represents the southern extent of the American black duck's (*Anas rubripes*) breeding range. Mallards (*A. platyrhynchos*) are present on the breeding grounds of the American black duck and hybridization is observed between these species; therefore, we assessed the genetic integrity, hybridization rates, and population structure of this local breeding population. We extracted genomic and mitochondrial DNA from chorioallantoic membranes and contour feathers from monitored black duck nests. We then prepared the extracted DNA for analysis using high-throughput DNA sequencing methods (ddRAD-seq). First, we assessed nuclear and mitochondrial population structure, genetic diversity, and differentiation across samples from North Carolina, and compared them against 199 genetically vetted mallards, black ducks, and mallard × black duck hybrids that served as genetic references. Next, we tested for parentage and sibling relationship and overall relatedness of black ducks in North Carolina. We recovered strong population structure and high co-ancestry across genetic markers due to interrelatedness among sampled nests in North Carolina and concluded that black ducks have been locally breeding in this area for a prolonged period of time. Despite a high level of interrelatedness among our samples, nucleotide diversity was similar to the reference continental black duck population, suggesting little effect of genetic drift, including inbreeding. Additionally, we conclude that molecular diversity of black ducks in North Carolina is maintained at reference population levels through the influx of genetic material from unrelated, migrating male black ducks. Finally, we report a hybridization level of 47.5%, covering 3 filial generations. Of identified hybrids, 54.7% and 53% were the direct result of interbreeding between black ducks and captive-reared or wild mallards, respectively. We conclude that because of high rates of interspecific hybridization and successive backcrossing events, introgression from wild and feral mallards is occurring into this population of breeding black ducks and requires careful consideration in future management efforts.

KEY WORDS *Anas platyrhynchos*, *Anas rubripes*, anthropogenic hybridization, breeding ecology, captive-reared mallard, population structure.

Although gene flow is a widespread evolutionary phenomenon across taxonomic lineages (Mallet 2007, Arnold and Kunte 2017), the increasing incidence of anthropogenic hybridization (i.e., hybridization due to human activity; Vilà et al. 2000) between taxa has important implications for conservation that vary temporally, spatially, and across biological systems (Arnold 1992, Barton 2001, Seehausen et al. 2008). Specifically, anthropogenic hybridization as a result of habitat change or the direct movement of individuals, is resulting in accelerated rates of interspecific interaction among closely related species (Vallejo-Marín and Hiscock 2016). Consequences of such events range from the breakup of co-adapted gene complexes (i.e., outbreeding depression) to the possibility of adaptive introgression (Arnold and Martin 2009). In extreme cases, prolonged or frequent events of introgression (i.e., gene flow between populations whose individuals hybridize, achieved when hybrids backcross to 1 or both parental populations; Rhymer and Simberloff 1996) can result in a hybrid swarm (McFarlane and Pemberton 2019) in which individual gene pools are eliminated, causing extinction in 1 or both parental taxa (Rhymer and Simberloff 1996, Simberloff 1996, Rhymer 2006, Seehausen 2006, Allendorf 2017). Effects of hybridization are further amplified when dealing with small populations, which are innately more susceptible to becoming genetically swamped (Wells et al. 2019). For

Received: 10 March 2020; Accepted: 20 March 2021

[1]*E-mail: dlawso@udel.edu*

example, on the Hawaiian Islands of O'ahu, Maui, Moloka'i, and Hawai'i, the Hawaiian duck (*Anas wyvilliana*) experienced extirpation by introgressive hybridization with introduced feral mallards (*Anas platyrhynchos*). Specifically, Wells et al. (2019) reported that the absence of large native Hawaiian duck populations and the establishment of feral mallards on these islands, advanced the formation of hybrid swarms and ultimately led to these extirpations. Thus, understanding the frequency and extent of introgressive hybridization among interacting species is increasingly important when managing populations (Allendorf et al. 2001, Randi 2008, McFarlane and Pemberton 2019).

Ducks and geese (Order Anseriformes) exhibit some of the highest levels of hybridization in class Aves (Johnsgard 1960, Scherer and Hilsberg 1982, Grant and Grant 1992). Notably in Anatinae ducks, the mallard readily hybridizes with many other Anatinae duck species including the American black duck (*A. rubripes*; black duck). Black ducks belong to a paraphyletic duck group with an estimated divergence time from mallards of 600,000 years before present (Lavretsky et al. 2019*a*). Prior to the 1950s, black ducks and mallards were mostly allopatric; however, increasing conversion of temperate forests to open land cover types in the breeding and wintering grounds of the eastern United States and Canada may have promoted mallard ranges to extend eastward (Livezey 1991, Green 1996, Johnson and Sorenson 1999, Mank et al. 2004). Additionally, some state agencies and private entities facilitated large-scale releases of captive-reared mallards, with 500,000 annually released along the east coast through the first half of the twentieth century (Heusmann 1974, Soutiere 1986, Hepp et al. 1988). More recently, an estimated 210,000 captive-reared mallards are being released annually in the Atlantic Flyway, with most releases occurring on licensed shooting preserves (U.S. Fish and Wildlife Service [USFWS] 2013). In general, these events resulted in an approximately 6-fold increase in mallard abundance east of the Mississippi River by the mid-1960s (Heusmann 1974, Soutiere 1986, Hepp et al. 1988), and have resulted in high rates of interspecific interactions with black ducks in eastern North America (Anderson et al. 1987, Ankney et al. 1987, Avise et al. 1990, Conroy et al. 2002, Lavretsky et al. 2019*b*). Moreover, the release of captive-reared mallards in eastern North America has resulted in an established feral (i.e., individuals of domestic origins but found in wild settings) and feral × wild mallard hybrid swarm (Lavretsky et al. 2019*c*). Thus, black ducks across eastern North America can interact with wild mallards and mallards of captive-reared origin.

Historically, the black duck was the most abundant duck species in the Atlantic Flyway, but because of population declines (>50%) between the 1950s and 1990s, black ducks are now a species of greatest conservation concern in 14 of 17 states (Devers and Collins 2011). Black ducks are also a flagship species of the Atlantic Coast Joint Venture and the North American Waterfowl Management Plan (Ringelman and Williams 2018). Potential explanations for their decline include overharvest (Nichols et al. 1987, Francis et al. 1998, Longcore et al. 2000), loss of breeding and non-breeding habitat quantity and quality (Conroy et al. 2002, Zimpfer 2004), and interactions (competition, hybridization) with mallards (Anderson et al. 1987, Conroy et al. 2002, Mank et al. 2004). Recent landscape-level sampling coupled with high-throughput DNA sequencing methods confirmed a high rate of hybridization of around 42% between mallards and black ducks (Lavretsky et al. 2019*b*), which is highest of all comparisons between mallards and other North American monochromatic Anatinae ducks (Lavretsky et al. 2015, Peters et al. 2016, Ford et al. 2017). Introgression of mallard genes into black ducks remained relatively low (Lavretsky et al. 2015, 2019*b*), suggesting that backcrossing of F1 (first filial generation) mallard × black duck hybrids into the black duck gene pool was somehow limited (e.g., assortative mating, hybrid-breakdown), and largely can be associated with lost reproductive potential. More concerning, however, was that many of the F1 hybrids and the few black duck-backcrossed samples recovered by Lavretsky et al. (2019*b*) showed nuclear genetic assignment to captive-reared mallards. These results suggest that much of the interspecific interaction that has been discussed between mallards and black ducks is the result of the established eastern North American feral mallard population (Lavretsky et al. 2019*b*). Additionally, mallards in North America carry 2 mitochondrial haplogroups; Old World (OW) A and New World (NW) B (Avise et al. 1990; Johnson and Sorenson 1999; Kulikova et al. 2004, 2005; Lavretsky et al. 2014). Lavretsky et al. (2019*c*) provided inferential evidence that the majority of OW A mitochondrial DNA (mtDNA) haplotypes in North America are due to gene flow from released captive-reared mallards. Thus, the possession of an OW A mtDNA haplotype is strong evidence of domestic mallard ancestry.

Determining genetic population structure is contingent on sampling genetic material of adequate quantity and quality (Taberlet et al. 1999). Although not the only option, blood is the most commonly sampled genetic material in avian genetic studies (Coulon et al. 2008, Höglund et al. 2009). Capture and handling techniques used to obtain avian blood samples can be invasive (Trimbos et al. 2009). In waterfowl, traditional blood-based sampling methods require capture of the nesting female or offspring to assess maternity and population structure during the breeding season. Disturbance associated with trapping and traditional sampling techniques can possibly compromise nesting success. A noninvasive alternative to sampling birds directly is the use of DNA-containing materials deposited by birds in nests (Pearce et al. 1997). Most waterfowl species deposit down and contour feathers in nests as incubation advances and chorioallantoic membranes often remain in the nest after hatch (Baldassarre 2014) providing researchers with alternate genetic materials. Previously, DNA has been successfully extracted and analyzed from feathers (Taberlet and Bouvet 1991, Ellegren 1992, Morin et al. 1994, Pearce et al. 1997) and chorioallantoic membranes (Pearce et al. 1997; Strausberger and Ashley 2001; Bush et al. 2005; Trimbos et al. 2009, 2014). Perceived limitations associated

with using noninvasive samples are chance contaminations with parental and sibling DNA (Taberlet and Fumagalli 1996, Taberlet and Waits 1998, Strausberger and Ashley 2001, Schmaltz et al. 2006), DNA degradation by ambient environmental conditions, and reduced DNA quantities compared to traditional samples (Pearce et al. 1997). Trimbos et al. (2009), however, presented inferential evidence that neither degeneration nor cross-contamination was apparent in total genotypic comparison of chorioallantoic membrane DNA and blood sample DNA. Thus, noninvasive materials (e.g., feathers, egg membranes) can provide a viable source of DNA for population genetic research.

In general, the black duck population in the Albemarle-Pamlico Peninsula and Outer Banks regions of coastal North Carolina, USA, is thought to be small, and largely composed of locally breeding individuals that rarely conduct long-distance movements (D. L. Howell, North Carolina Wildlife Resources Commission, personal communication). Although genetic drift disproportionally effects populations of small size (Wang and Caballero 1999), inbreeding may also be a particular issue for ducks that exhibit seasonal monogamy and female nest-site fidelity (Coulter and Miller 1968). Thus, we endeavored to understand whether any observable population structure was due to sibling relationship, inbreeding, or both.

Our objectives were to assess the genetic integrity and population structure of a black duck population at the southernmost extent of their breeding range and to measure the relationship among samples (i.e., sibship, parentage) to determine the extent to which interrelatedness is present in the black duck population in North Carolina. Because captive-reared mallard releases occur in eastern North Carolina, we predicted that most hybrids would be of feral mallard × black duck ancestry. We predicted to see multiple filial generations of hybrids if hybrids readily backcrossed. Alternatively, if hybrid survival was low or selected against during pair bonding, then we predicted to find little evidence of backcrossed hybrids. Finally, if the North Carolina breeding black duck population is in immediate risk of inbreeding depression, we predicted to find low genetic structure, high levels of interrelatedness, and low haplotype and nucleotide diversity within black ducks in North Carolina as compared to reference black duck populations.

STUDY AREA

We sampled black duck and mallard nests in Hyde and Dare counties in the Albemarle-Pamlico Peninsula and the Outer Banks regions of North Carolina from late March to the end of June in 2017 and 2018 (Fig. 1). This area represents the southernmost extent of the black duck's breeding range in the Atlantic flyway (Parnell and Quay 1962,

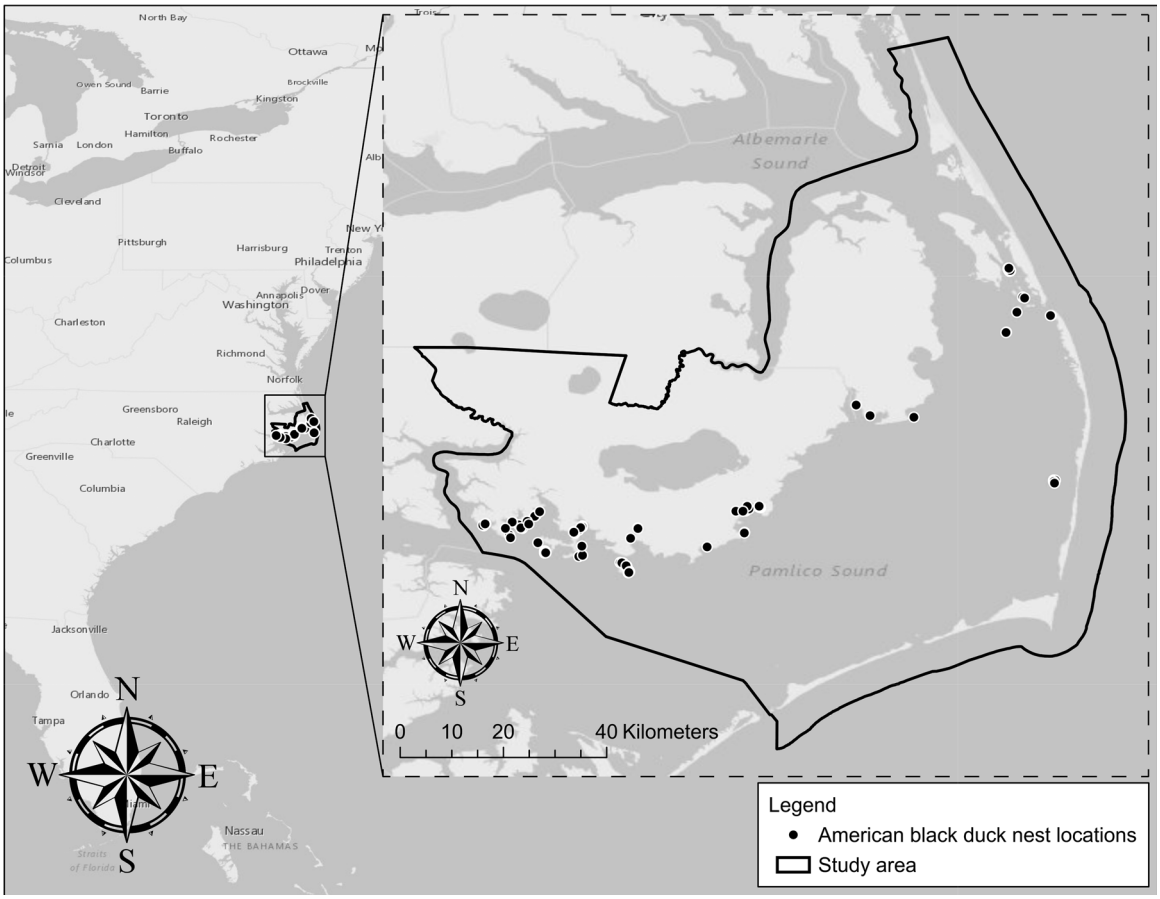

Figure 1. Study area of Hyde and Dare counties, North Carolina, USA, 2017–2018, and sampling locations for American black duck nests.

Bellrose 1980, Baldassarre 2014). Specifically, our research focal area covered approximately 5,562 km^2 and ranged from the convergence of the Pamlico and Pungo rivers northeast along the Pamlico Sound to the intersection of United States Highway 64 and the Croatan Sound and throughout the Outer Banks of Hyde and Dare counties. The area is relatively flat with an elevation averaging <1.5 m above mean sea level. The climate is characterized by hot summers (Jun–Aug) and cool winters (Dec–Feb). Temperatures in July average 26°C and in January average 6°C. Total annual precipitation averages 132 cm, with approximately 50% occurring during May–September. The dominant land use is for agriculture, forestry, and tourism. Study sites included Swanquarter National Wildlife Refuge (NWR; 35°21′59.99″N, −76°19′18.00″W), Alligator River NWR (35°46′59.99″N, −75°50′59.99″W), Pea Island NWR (35°41′17.99″N, −75°32′26.99″W), and private and state-owned lands along the Pamlico Sound.

The Albemarle-Pamlico Peninsula historically was dense in wetlands, but most of the wetlands were drained and converted to agricultural cropland. Existing wetlands include large natural lakes, river drainage systems of the Alligator, Pungo, and Long Shoal rivers, large contiguous blocks of semi-permanently flooded swamp, pocosins, and freshwater and brackish marshes. The Outer Banks region is a barrier island complex bordered by Pamlico, Croatan, and Core sounds to the west and the Atlantic Ocean to the east. This area is dominated by extensive brackish and saltmarsh, beach dunes, maritime shrub and dry grassland, and various early successional cover types, including man-made dredge spoil islands. Dominant vegetation at nesting sites within brackish and saltmarshes included black needlerush (*Juncus roemerianus*), salt meadow cordgrass (*Spartina patens*), and saltgrass (*Distichlis spicata*), whereas those on dredge spoil islands were found most often in dense vegetation dominated by warm-season grasses, blackberry (*Rubus* spp.), and forbs. Dominant fauna included raccoon (*Procyon lotor*), mink (*Neovison vison*), American crow (*Corvus brachyrhynchos*), fish crow (*Corvus ossifragus*), bald eagle (*Haliaeetus leucocephalus*), American black bear (*Ursus americanus*), and various species of waterfowl and waterbirds.

METHODS

Over the 2017 and 2018 nesting seasons (i.e., Mar–Jul), we systematically searched for black duck nests in areas that North Carolina Wildlife Resources biologists identified as suitable for nesting, as determined via breeding black duck surveys (D. L. Howell, unpublished report). We opportunistically sampled mallard nests throughout the same time period. To search for nests, we hand dragged a 30.5-m rope across potential nest locations. We attached aluminum cans with rocks inside at 2-m intervals along the entire length of the rope to serve as noisemakers (modified from all-terrain vehicle chain drags used for upland nesting waterfowl; Klett et al. 1986). In areas where vegetation height and rigidity hindered us from using rope drags, we distanced 5–25 m apart and walked transects of the search area. We conducted nest searches for both species in 2017 systematically and then we implemented more-focused searches in 2018. To increase nest sample size, we prioritized our efforts after developing a search image of nesting habitat cues. We checked nests every 7–10 days until the nest was terminated (i.e., successful, abandoned, destroyed, nonviable, unknown), either when the female was located away from the nest site or by flushing the female from the nest. Nest searching and monitoring methods followed Guidelines to the Use of Wild Birds in Research (Fair et al. 2010).

Once monitored nests for both species were terminated, we collected all chorioallantoic membranes and ≥10 maternal contour feathers from the nest bowl. We labeled and stored samples at room temperature in separate plastic bags. We attempted DNA extraction and isolation on all collected chorioallantoic membranes and maternal contour feather calami to have at minimum 1 representative duckling and maternal sample from each sampled nest. We extracted and isolated DNA using a Qiagen DNAeasy blood and tissue kit (Qiagen, Valencia, CA, USA) and following manufacturer's protocols. We quantified extractions using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to ensure a minimum concentration of 0.02 μg/μl.

ddRAD-seq Library Preparation and Bioinformatics

We prepared multiplexed double digest restriction-site associated DNA (ddRAD-seq) fragment libraries following Lavretsky et al. (2015*a*). We then pooled samples in equimolar concentrations and completed 150 base pair (bp), single-end sequencing on an Illumina HiSeq. 4000 (Illumina, San Diego, CA, USA) at the University of Oregon's Genomics and Cell Characterization Core Facility. All Illumina raw reads are deposited in the National Center for Biotechnology Information's Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra, accessed 24 Sep 2018; BioProject PRJNA699265, accession numbers SAMN17770951–SAMN17770990). In addition to our samples, we included previously published ddRAD raw sequence data from Lavretsky et al. (2019*b*) for 199 black ducks ($n = 46$), wild mallards ($n = 35$), mallard × black duck F1 hybrids ($n = 21$), and captive-reared mallards from game farms in New Jersey and Kentucky, USA ($n = 48$). These samples served as references of each population for comparative purposes to our black duck population in North Carolina regarding population structure and nucleotide diversity.

Mitochondrial DNA Sequencing and Alignment

We used Primers L78 and H774 (Sorenson and Fleischer 1996, Sorenson et al. 1999) to amplify and sequence 625 bp of the mtDNA control region following protocols described in Lavretsky et al. (2014). All products were Sanger sequenced 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 are deposited in GenBank (accession numbers MW574482–MW574589).

Determining Population Structure and Molecular Diversity among Samples

We used a dataset of independent bi-allelic autosomal ddRAD-seq single nucleotide polymorphisms (SNPs), with singletons removed across analyses of population structure. We completed all analyses without *a priori* information on population or species identity. First, we used the program PLINK version 1.07 (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 assessed independence among SNPs through pair-wise linkage disequilibrium tests across ddRAD-seq autosomal SNPs, with 1 of 2 SNPs randomly excluded if a linkage disequilibrium correlation factor (r^2) > 0.5 was obtained.

First, we analyzed clustering among samples using the dudi.pca function in the R Statistical software (R Core Team 2020) package Adegenet (Jombart 2008) to perform a principal component analysis (PCA). Next, we calculated a matrix of co-ancestry coefficients based on the distribution of identical or nearest neighbor haplotypes among samples with the program fineRADstructure (Malinsky et al. 2018). In short, recent co-ancestry is emphasized by rare SNPs (Kimura and Ohta 1973), and thus, an increase in these SNPs corresponds with relatedness. 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. We visualized results with the R scripts fineradstructureplot.r and finestructurelibrary.r (http://cichlid.gurdon.cam.ac.uk/fineRADstructure.html, accessed 24 Sep 2018).

Finally, we calculated maximum likelihood individual assignment probabilities with the program ADMIXTURE version 1.3 (Alexander et al. 2009, Alexander and Lange 2011). We ran ADMIXTURE analyses with a 10-fold cross-validation, and with a quasi-Newton algorithm employed to accelerate convergence (Zhou et al. 2011). To limit any possible stochastic effects from single analyses, we ran 100 iterations at each population *K*-value, which indicates the number of genetic clusters inferred (ranging from 1–10). Each analysis used a block relaxation algorithm for point estimation and terminated once the change (i.e., delta) in the log-likelihood of the point estimations increased by <0.0001. We based the optimum *K* on the average of coefficient of variation errors across the 100 analyses per *K*; however, we analyzed additional *K*s for further population structure resolution. After using the R program PopHelper (Francis 2017) to convert ADMIXTURE outputs into CLUMPP input files, we used the program CLUMPP version 1.1 (Jakobsson and Rosenberg 2007) to determine the robustness of the assignments of individuals to populations at each *K*. We employed the large greedy algorithm and 1,000 random permutations, with final admixture proportions for each *K* and per sample assignment probabilities (Q estimates; the log likelihood of group assignment) based on CLUMPP analyses of all 100 replicates per *K* (Table S1, available online in Supporting Information). We used per-sample assignment probabilities to assign samples to black ducks, wild mallards, feral mallards, and mallard × black duck hybrids (first through third filial generations and direction of backcross) following purity thresholds defined by Lavretsky et al. (2019*b*). Specifically, we applied 6 purity thresholds per Lavretsky et al. (2019*b*) to classify individuals with 1) ≥95% black duck assignment as pure black ducks, 2) ≥98% mallard assignment as pure (wild or feral) mallards, 3) 27–72% interspecific assignment as F1 hybrids, 4) 10–27% as F2-black duck backcrosses, 5) 2–27% black duck assignment as F2-mallard backcrosses, and 6) 5–10% mallard assignment as F3-black duck backcrosses.

For mtDNA analyses, we included North Carolina samples and an additional 199 genetically vetted wild mallards, captive-reared mallards, black ducks, and F1 black duck × mallard hybrids (Table S2, available online in Supporting Information; GenBank accession numbers MK425222–MK425495 from Lavretsky et al. 2019*b*). We visualized population structure via a haplotype network reconstructed in the program Network version 5 (Bandelt et al. 1999; fluxus-engineering.com, accessed 25 Sep 2018), which we also used to determine samples carrying OW A versus NW B mtDNA haplotypes. We used mtDNA sequences from offspring samples to determine the maternal lineage in cases where sequencing of the maternal sample failed. Finally, we calculated composite pair-wise population estimates of relative differentiation ($\Phi_{ST;}$ Hudson et al. 1992), and per population nucleotide diversity (π; Hudson et al. 1992, Wakeley 1996) across mtDNA and ddRAD-seq loci in the R package PopGenome (Pfeifer et al. 2014).

Interrelatedness among North Carolina Samples

We quantified sample relatedness using the program COLONY version 2.0.6.5 (Jones and Wang 2010). Program COLONY implements full-pedigree likelihood methods to simultaneously infer sibship and parentage among individuals using multilocus genotype data. Analyses in COLONY were based on ddRAD-seq autosomal loci with <5% missingness and a minimum allele frequency of 0.5 across samples. To reduce the risks of type I error, we only reported parental, full-sibling, and half-sibling dyads with pairwise relatedness estimates that were greater than 0.2 (Lebigre et al. 2010). Additionally, we used the half-sibling dyads to infer quarter sibling (i.e., second cousins) relationships. Any female whose lineage had successfully bred in the area for generations would show a substantial number of quarter siblings as a result of their nieces and nephews also successfully breeding. In contrast, any non-related females breeding for the first time in the area would have been and would have had offspring with no direct relationships to individuals from other nests. In general, if few migrants moved into the North Carolina population, we expected to find substantial interrelatedness given the hypothesis that the local breeding population is small and likely disjunct from the main black duck population

breeding in eastern Canada and the mid-Atlantic and northeastern United States (D. L. Howell, personal communication).

RESULTS

We pooled data across years to assure adequate sample size and made the assumption that a year effect did not exist. We achieved sufficient DNA quality and quantity required to construct ddRAD-seq libraries for 35% (32 of 92) of duckling chorioallantoic membranes and 21% (8 of 39) of maternal contour feathers, representing genomes at 40 separate nests. After combining all successfully sequenced samples and those acting as reference populations, we obtained a dataset of 1,330 ddRAD-seq autosomal loci (195,984 bp; 22,155 polymorphisms) that met our coverage and missing data criteria. Our final datasets contained loci with an average of 94% of alleles per individual being present, ensuring that data missingness was not a problem in downstream analyses.

Nuclear Population Structure and Interrelatedness of North Carolina Samples

Population structure analyses were based on 7,997 (of 8,737) independent bi-allelic ddRAD-seq autosomal SNPs that matched our filtering criteria. First, we recovered an optimum *K* population of 2 in ADMIXTURE analyses where captive-reared mallards were clearly distinguished from all others; however, additional resolution of population structure was achieved up to a *K* population of 4 (Fig. S1, available online in Supporting Information). At a *K* population of 4, we were able to identify previously described structure between captive-reared mallards, wild mallards, and parental black duck samples (Lavretsky et al. 2019*b*) and recover a unique genetic structure within many of the samples from North Carolina (Fig. 2A). The same 4 population units were also recovered in fineRADstructure co-ancestry plots (Fig. 2B); the highest co-ancestry assignments were recovered among North Carolina samples that made up the North Carolina genetic cluster in ADMIXTURE analyses (Fig. 2A). Finally, plotting the first 3 principal components identified 3 primary groups consisting of our reference captive-reared mallards, wild mallards, and American black ducks, whereas North Carolina samples were largely scattered among, within, and around these groups (Fig. 2C).

Using 262 ddRAD-seq autosomal SNPs that met our coverage and minimum allele frequency requirements to quantify relatedness among samples in the program COLONY, we detected 32 parentage assignments, 5 full-sib pairings, and 38 half-sib pairings with pairwise relatedness estimates that were greater than 0.2 (Fig. S1). We inferred an additional 67 quarter sibling pairings (i.e., second cousins) from the half sibling relationships that demarcated several clusters of related lineages. Among them, we identified 2 females (i.e., 127C and 058C) that had a number of half- and quarter-sibling relationships suggesting a minimum of 3 generations of breeding cohorts overlapping in space and time. All samples assigned to the North Carolina genetic group in ADMIXTURE (Fig. 2A) and those that had the highest co-ancestry (Fig. 2B) were identified as highly interrelated in the program COLONY (Fig. S2). Additionally, we clearly recovered the 2 sets of full siblings in fineRADstructure (Fig. 2B) and PCA analyses (Fig. 2C). Thus, the unique genetic cluster of black ducks in North Carolina was best explained by high interrelatedness. We also conclude that the distortion in the clustering of North Carolina samples within the PCA analysis was a product of their interrelatedness (Wang 2018, O'Connell et al. 2019). Together, for North Carolina samples, we assigned 30% (12 of 40) as related black ducks, 13% (5 of 40) as second cousin black ducks, 20% (8 of 40) as unrelated black ducks (i.e., unrelated to the North Carolina population), 15% (6 of 40) as putative feral mallard × black duck hybrids, and 23% (9 of 40) as putative wild mallard × black duck hybrids (Table 1). Moreover, we categorized 52.5% (21 of 40) of these samples as pure black ducks and 47.5% (19 of 40) as hybrids, and categorized 53% (10 of 19) and 47% (9 of 19) of hybrids as resulting from mate pairings between a black duck and either a wild or captive-reared mallard, respectively. Captive-reared genetic assignment recoveries occurred in relation to captive-reared mallard release areas (Fig. S3, available online in Supporting Information); however, we did not detect an obvious spatial relationship between these data.

Finally, the apparent interrelatedness of our North Carolina samples complicated ADMIXTURE analyses and our ability to properly assign samples to late-stage hybrid classes (≥F3). In fact, we detected how relatedness can bias ADMIXTURE analyses after considering the near-perfect interspecific assignments observed across individuals comprising the related black duck genetic clusters from North Carolina and game-farm mallards from Kentucky and New Jersey (Fig. 2A). This indicates that results were influenced by strong familial structure, as evident in the fineRADstructure co-ancestry plot for these exact groups (Fig. 2B). Together, we conclude that the high co-ancestry largely hinders our capacity to be confident in the identification of ≥F3 stage hybrids (i.e., 5–10% mallard assignment as F3-black duck backcrosses). For example, 2 related black duck samples had higher than average co-ancestry assignment to captive-reared mallards but low assignment probability to them in ADMIXTURE analyses (i.e., ~2% assignment to captive-reared mallard; Fig. 1A). But when we compared known hybrids to samples in the 2 North Carolina hybrid clusters, we recovered similar interspecific assignment probabilities, mixed co-ancestry scores, and intermediate clustering in our PCA (Fig. 2). Most evident were samples that were identified as being captive-reared mallard × black duck hybrids because they were found in intermediate space in PCA, equal assignment probabilities, and co-ancestry assignment to the 2 parental populations (Fig. 2). Together, we conclude that the inclusion of all 3 analyses provides resolution where relatedness may bias other analyses (O'Connell et al. 2019) and are confident in identifying hybrids, including assignment of hybrids up to the F2-backcross group. In the end we determined that 47%

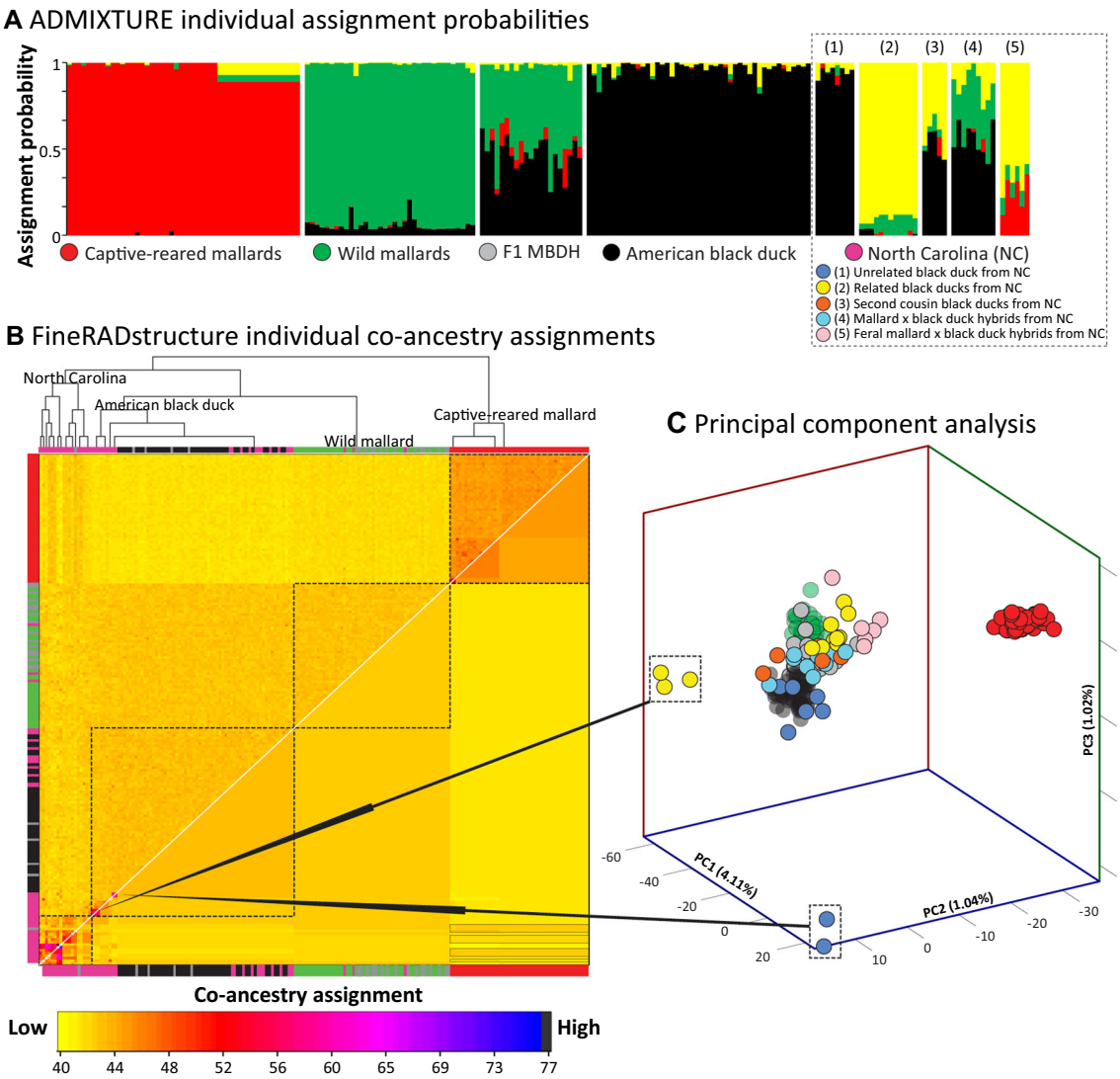

Figure 2. A) Individual assignment probabilities as estimated in the program ADMIXTURE (Alexander et al. 2009) at a population *K* of 4, B) co-ancestry estimates from fineRADstructure, and C) plotting of the first 3 principal components (PCs) from our principal component analysis across American black ducks, wild mallards, captive-reared mallards, and mallard × American black duck hybrid (MBDH) samples and based on a 7,997 independent bi-allelic double digest restriction-site associated DNA (ddRAD-seq) autosomal single nucleotide polymorphism (SNP) dataset. North Carolina samples are broken up into 5 genetic classes, and with all groups color coded throughout analyses.

and 42% of hybrids had assignment probabilities within the expected ranges of F1 and F2-black duck backcrossed hybrids, respectively.

Genetic Differentiation and Diversity

Within North Carolina samples, related black ducks were genetically differentiated ($\Phi_{ST} = 0.06$) from unrelated black ducks (Fig. 3). Moreover, comparisons of North Carolina and reference samples of mallards yielded differentiation estimates of 0.05 for related black ducks and 0.025 for unrelated black ducks. Similarly, estimated differentiation from reference black ducks was 0.05 for related black ducks and 0.01 for unrelated black ducks. Finally, genetic differentiation was apparent for related black ducks and unrelated black ducks, ($\Phi_{ST} = 0.12$ and 0.11, respectively) when compared against known captive-reared mallards. Collectively, differentiation estimates between all North Carolina breeding black duck samples and reference mallards and black ducks was 0.019, and 0.09 from known captive-reared mallards (Fig. 3). Despite the high interrelatedness and a moderate inbreeding coefficient of 0.105, π was 0.0070 in related and 0.0072 in unrelated North Carolina sample black ducks, and was comparable to reference black ducks and mallards ($\pi \sim 0.0071$; Fig. 4).

Mitochondrial Diversity and Population Structure

We successfully amplified 624 base-pairs of the mtDNA control region and sequenced across 82% (75 of 92) of duckling chorioallantoic membranes and 85% (33 of 39) of maternal contour feathers, representing 108 mtDNA genomes at 93 separate nests. Previously described OW A and NW B mtDNA haplogroups (Avise et al. 1990, Johnson and Sorenson 1999, Kulikova et al. 2005, Lavretsky

Table 1. Simulation-based indices for pure black ducks (ABDU), F1 hybrids (MBDX F1), F2-black duck backcrosses (ABDX F2), and F3-black duck backcrosses (ABDX F3). Per the index, assignment probabilities are based on the proportion (prop.) of intra- and inter-specific assignment. Purity assignments are based on percentage assigned to black duck populations, coastal North Carolina, USA, 2017–2018. The number of samples with Old World A mitochondrial (mtDNA) haplotypes for each index are noted.

Group	Index	Estimate	*n*
American black duck	Pure ≥95%	22 (0.55)	22
	Prop. assigned to feral mallard group	0	
	Prop. A mtDNA haplogroup	0	
Hybrid (MBDX F1)	27% < F1 ≤ 72%	10 (0.25)	10
	Prop. assigned to feral mallard group	4 (0.40)	
	Prop. A mtDNA haplogroup	0	
F2 toward ABDU (ABDX F2)	10% < F2 ≤ 27%	6 (0.15)	6
	Prop. assigned to feral mallard group	4 (0.64)	
	Prop. A mtDNA haplogroup	1 (0.03)	
F3 toward ABDU (ABDX F3)	5% < F3 ≤ 10%	2 (0.05)	2
	Prop. assigned to feral mallard group	2 (1.00)	
	Prop. A mtDNA haplogroup	0	

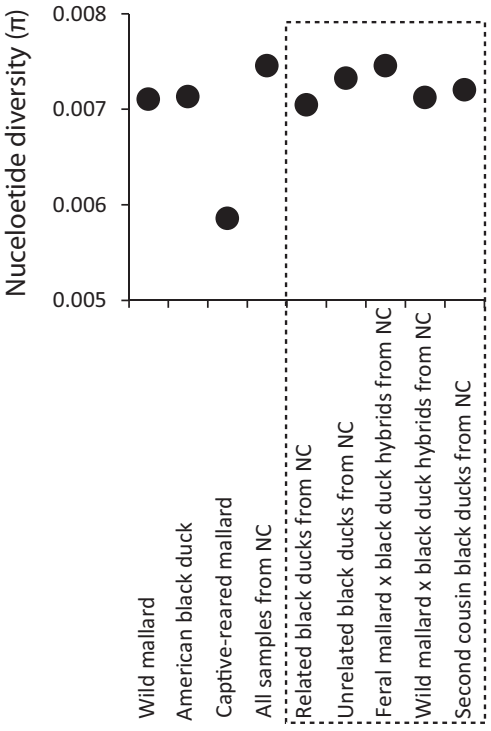

Figure 4. Nucleotide diversity (π) estimated from 1,330 nuclear double digest restriction-site associated DNA (ddRAD-seq) loci among reference mallard, black duck, and captive-reared mallard samples, and against the 5 genetic populations identified in North Carolina, USA, 2017–2018.

et al. 2019*a*) were recovered in the mtDNA haplotype network (Fig. 5) and represented spatially in relation to captive-reared mallard release sites (Fig. S4, available online in Supporting Information). Of phenotypically- and genetically assigned black ducks, mallards, and their hybrids, 8 of 88, 3 of 5, and 1 of 15 possessed OW A haplotypes, respectively (Table S2). Remaining black ducks (80 of 88), mallards (2 of 5), and genetically assigned mallard × black duck hybrids (14 of 15) possessed NW B haplotypes (Table S2).

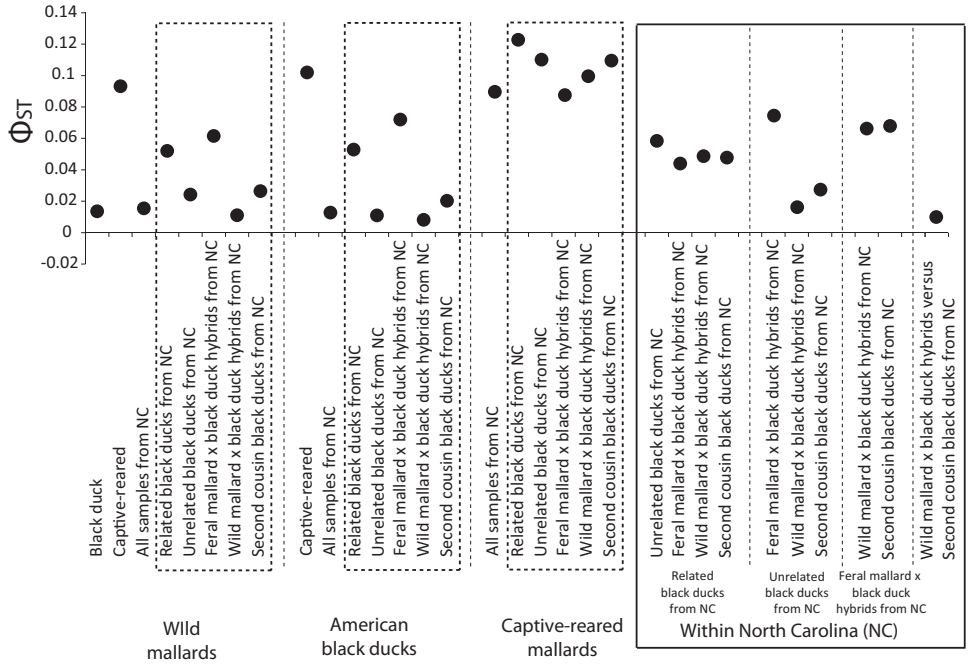

Figure 3. Pair-wise estimates of differentiation (Φ_{ST}) from 1,330 nuclear double digest restriction-site associated DNA (ddRAD-seq) loci and between genetically vetted mallards, American black ducks, and captive-reared mallards, and the 5 genetic populations identified in North Carolina, USA, 2017–2018. In addition, we provide within-North Carolina comparisons denoted in the solid box.

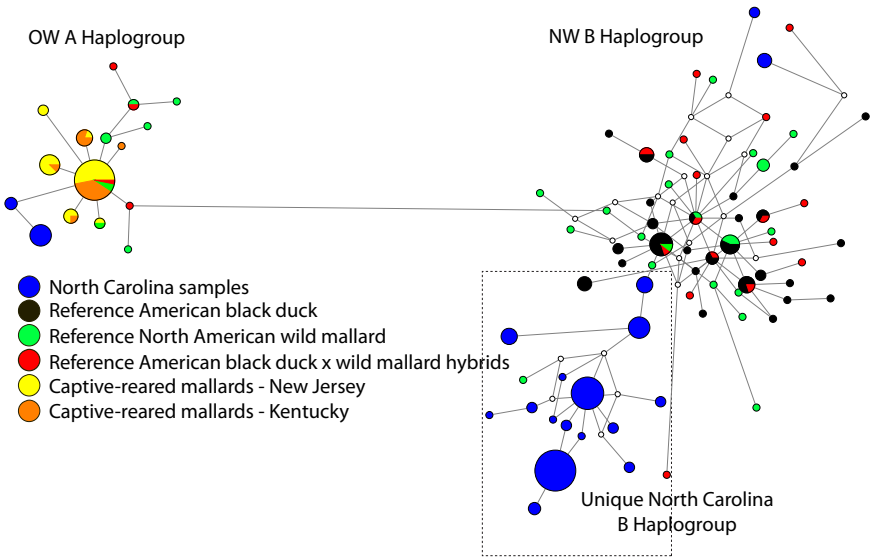

Figure 5. Reconstructed haplotype network for the mitochondrial DNA (mtDNA) control region sequenced for reference American black ducks, mallards, captive-reared mallards, and hybrids, and samples obtained from North Carolina, USA, 2017–2018. Graduated circles represent sample size and network lines represent genetic distance between and within mitochondrial haplogroups. The previously identified Old World A (OW A) and New World B (NW B) haplogroups and the unique North Carolina haplogroup nested within the NW B cluster are denoted. With the exception of 1 mallard, the remaining recovered haplotypes within the North Carolina haplogroup were all specific to North Carolina samples.

DISCUSSION

High Interrelatedness and Localized Breeding Explains Population Genetics

Molecular assessment of a locally breeding black duck population in North Carolina revealed unique genetic structure largely explained by high interrelatedness among sampled individuals. Specifically, we recovered nuclear (Fig. 2) and mtDNA (Fig. 5) structure within North Carolina samples that were evidently different from reference populations, including continental black ducks. In addition to having some of the highest levels of co-ancestry (Fig. 2C), most black ducks in North Carolina possessed a specific NW B mtDNA haplogroup. Given that mtDNA is maternally inherited and Anatinae waterfowl exhibit female-biased natal and breeding philopatry (Coulter and Miller 1968, Greenwood and Harvey 1982, Clarke et al. 1997, Zhuravlev and Kulikova 2014), the recovery of this unique cluster of haplotypes within the NW B haplogroup suggests localized population structure in the area and specifically among breeding females. Among black ducks, 36% of adult females are philopatric to natal breeding sites, and in extreme cases have been reported to repeatedly nest within the same wetland (i.e., <0.25 km away annually; Reed 1975, Ringelman et al. 1982, Seymour 1991). Inferring from our nuclear- and mtDNA-based analyses (Figs. 2, and 5), we recovered 2 renesting females, 3 returning females, and 2 breeding sisters that nested <0.05 km from previous nests of one another. Thus, the unique cluster of mtDNA haplotypes and recovery of related females breeding close to one another is consistent with the philopatric nature of these birds. Finally, given the number of mutations that have arisen in the mtDNA haplogroup of black ducks in North Carolina (Fig. 5) and clusters of individuals with half and quarter sibling (Fig. S2, available online in Supporting Information), relatives across different nests also suggests that many of these lineages have been breeding in North Carolina for some time. In fact, the 2 females (i.e., 058C, 127C) with multiple half- and quarter-sibling relationships with sampled eggs from different nests (Fig. S2) demonstrates their lineage's breeding propensity and the evident generational overlap of breeding females in the area, in general. Although we contend that the geographical space that was sampled is representative of breeding black ducks in the sampled coastal marshes (Fig. 1), future work will benefit from increased sampling of interior North Carolina and of temporal sampling of the current locations to further confirm how much of this black duck population is composed of the same, perpetuated lineages.

Despite strong population structure due to elevated levels of interrelatedness, nucleotide diversity remained high ($\pi \geq 0.007$) and similar to reference populations. Among our samples, we recovered 8 unrelated black ducks that were genetically most similar to the reference black ducks in our dataset, which indicates non-residents do breed in North Carolina. Thus, despite the potential for bottlenecking due to founder events or inbreeding, we posit that the influx of genetic material from non-resident birds via gene flow likely maintains high molecular diversity in North Carolina. Males are the dispersing sex in ducks (Rohwer and Anderson 1988) and most black ducks in the North Carolina sample had a maternal lineage of mtDNA haplotypes only found in North Carolina, which suggests that the majority of interbreeding is likely with immigrating male black ducks (Cooke et al. 1975). Conversely, we identified several breeding females that did not show high co-ancestry (Fig. 1C) or relatedness with other sampled females and nests (Fig. S1). Together, we conclude that the influx of genetic diversity is contributed by immigrating

males and females. Because nucleotide diversity in this local black duck population is comparable to reference populations, we conclude that further bottlenecking, including inbreeding is not currently an immediate risk to the black duck population in North Carolina.

Hybridization Rates in North Carolina

Based on nuclear assignment, 52.5% of samples were characterized as pure black ducks; the 47.5% hybridization rate recovered here is comparable to those reported for the continental population of black ducks (~42%; Lavretsky et al. 2019*b*). This rate, however, remains higher than for other North American monochromatic mallard-like taxa (e.g., ~2–5% hybridization rate between mallards and Mexican [*A. diazi*] or mottled [*A. fulvigula*] ducks; Lavretsky et al. 2015, Peters et al. 2016, Ford et al. 2017). Most hybrid samples we recovered had feral mallard contributions apparent in 3 filial generations. Additionally, the 11% of mtDNA samples that we recovered with OW A haplotypes were the direct result of gene flow from female feral or captive-reared mallards in North America, as mtDNA is maternally inherited and OW A haplotypes are of captive-reared mallard origins (Lavretsky et al. 2019*c*). Of the 4 mallard nests opportunistically sampled in our study area, we identified 2 genetically (based on mtDNA) as feral female mallards that were nesting in similar areas as black ducks. This finding further confirms that captive-reared mallards are fully capable of using breeding habitat outside of their originating preserves and do therefore become feral. Alternatively, of the 23% (9 of 40) of North Carolina samples that we found with recent (i.e., within 4 generations or ≥5% assignment) nuclear contributions from feral mallards, none possessed OW A haplotypes. This can only occur when female black ducks or hybrids with NW B haplotypes copulate with male mallards or hybrids of captive-reared ancestry (Lavretsky et al. 2019*b, c*). Thus, we conclude that although female feral mallards are breeding in eastern North Carolina marshes, most of the black duck × feral mallard introgression is the result of gene flow from male feral mallards. Whether these interspecific mating events are from the direct release of captive-reared mallards in eastern North Carolina (~15,000 annually; J. C. Fuller, North Carolina Wildlife Resources Commission, personal communication), or releases within the remaining Atlantic Flyway states (>80,000 free-flight releases annually; USFWS 2013) remains unknown. The 47.5% hybridization rate recovered in this study does suggest that these interspecific mating events pose some threat to breeding black ducks in North Carolina. In addition to decreasing molecular diversity, the movement of maladaptive domestic traits into wild populations can result in outbreeding and decreased adaptive potential of those wild populations. The negative effects of frequent interbreeding between domestic and wild types often takes several generations (i.e., hybrid breakdown) to be observed (Latta et al. 2007, Ellison and Burton 2008, Arcella et al. 2014, Stelkens et al. 2015, Bolstad et al. 2017). Thus, understanding the frequency and extent of anthropogenic hybridization among interacting species is increasingly critical when attempting to manage populations (Allendorf et al. 2001), particularly when dealing with small populations where the effects of hybridization are often amplified (Wells et al. 2019).

Despite having a high rate of hybridization and being relatively small and isolated, pure black ducks remain in North Carolina; therefore, the observed hybridization is not characteristic of a hybrid swarm (McFarlane and Pemberton 2019). This suggests that introgression or gene flow is still, to some extent, limited. In addition to the potential that mallard (wild or feral) × black duck hybrids may be less adaptive on the landscape (i.e., Haldane's Rule, Kirby et al. 2004; or hybrid breakdown, Dobzhansky and Dobzhansky 1971, Armbruster et al. 1997, Galloway and Fenster 1999, Hall and Willis 2005), assortative mating may also be resulting in low levels of incorrect mate pair selection, including mating with hybrids by black ducks. Alternatively, the mispairings may simply be due to extra-pair or forced copulation events that mallards are known to display (Barrett 1973, Mineau et al. 1983, Seymour 1990, Davis 2002), especially those of domesticated origins (Burns et al. 1980; Cheng et al. 1982, 1983). Under such a scenario, we posit that annual hybridization rates in North Carolina are simply a direct proportion of wild or feral mallards on the landscape that can potentially mate each year. Future research will generally benefit from investigating the number of mallards breeding in the area, and the change in feral mallard × black duck pairing events through time.

The intent of Federal Code (§ 21.13 of Title 50, 1975) was to provide privately operated shooting preserves unlimited opportunity to shoot captive-reared mallards. A 2013 review by USFWS stated that there is sufficient ambiguity in the regulation as it relates to the method of release and containment of captive-reared mallards to the area of the shooting preserve to consider amending it or to promulgate actions which further restrict intermixing of captive-reared mallards with wild migratory waterfowl. Further, it is worth noting that this Federal Code was last amended in 1989 when our knowledge of genetic introgression was not as well developed as it is today. Therefore, because no further action has occurred, there continues to be an unlimited potential for spatial mixing and genetic swamping of wild birds.

Considerations when Noninvasively Sampling Avian Nests

Noninvasive sampling for molecular research often contends with highly degraded DNA due to various factors (e.g., sunlight, moisture; Pearce et al. 1997) that can result in unsuitable DNA for some partial-genome sequencing methods that require intact enzymatic cut-sites to be present (e.g., ddRAD-seq; DaCosta and Sorenson 2014). Our 35% success rate in creating working ddRAD sequencing libraries is similar to other studies using noninvasive methods (Taberlet et al. 1999, Perry et al. 2010, Hans et al. 2015, Janjua et al. 2019), and attests to these limitations. In addition to environmental degradation of DNA, contour feathers and chorioallantoic membranes often have low quantities of DNA that become problematic for many

next-generation sequencing methods requiring relatively high amounts of starting DNA (Van Dijk et al. 2014). Nevertheless, increasing proportional success when applying ddRAD sequencing methods on such noninvasively obtained tissue samples would benefit from halting any further DNA degradation by immediately flash-freezing and storing samples at −80°C (Wong et al. 2012), and collecting more noninvasive materials per sample (e.g., >20 contour feathers vs. ≥10) to obtain sufficient amounts of DNA.

Despite the relatively low success in using outlined ddRAD sequencing methods, the declining costs and time associated with obtaining such data allows researchers to process many samples at once for thousands of markers and obtain large enough datasets to answer important population questions. Specifically, our sample dataset was large enough to capture ≥95% of the local nesting black duck population's genetic diversity (Nazareno et al. 2017, Leipold et al. 2020). Moreover, simulations across studies established that late generation hybrids into the F7 backcross stage are accurately assessed when using >1,000 loci (Boecklen and Howard 1997, Lavretsky et al. 2019*c*, Caniglia et al. 2020, Leipold et al. 2020). Although we demonstrate the limitations posed by strong familial structure on ADMIXTURE and PCA analyses, coupling these with co-ancestry assignments and sibship analyses allows researchers to make robust inferences into population structure and diversity, and provides confidence in assigning purity levels across sampled individuals (Wang 2018, O'Connell et al. 2019).

MANAGEMENT IMPLICATIONS

Feral mallards of captive-reared origin are intermixing with black ducks in North Carolina. Therefore, in its current state this population of breeding black ducks is at risk of incurring negative genetic consequences (e.g., genetic swamping, decreased molecular diversity, reduced adaptive potential) from prolonged anthropogenic hybridization with feral mallards.

ACKNOWLEDGMENTS

We thank P. R. Krausman, J. N. Straub, and 2 anonymous reviewers for their helpful editorial suggestions. We appreciate support from the University of Delaware College of Agriculture and Natural Resources, the University of Texas, El Paso Department of Biological Sciences, and the Pea Island, Swan Quarter, and Alligator River National Wildlife Refuges. Funding for this research was provided by the North Carolina Wildlife Resources Commission, through its State Waterfowl Stamp Fund, and the University of Delaware, Bombay Hook–Turners Creek graduate fellowship.

LITERATURE CITED

Alexander, D. H., and K. Lange. 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics 12:246.

Alexander, D. H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research 19:1655–1664.

Allendorf, F. W. 2017. Genetics and the conservation of natural populations: allozymes to genomes. Molecular Ecology 26:420–430.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16:613–622.

Anderson, D. R., K. P. Burnham, J. D. Nichols, and M. J. Conroy. 1987. The need for experiments to understand population dynamics of American black ducks. Wildlife Society Bulletin 15:282–284.

Ankney, C. D., D. G. Dennis, and R. C. Bailey. 1987. Increasing mallards, decreasing American black ducks: coincidence or cause and effect? Journal of Wildlife Management 51:523–529.

Arcella, T. E., W. L. Perry, D. M. Lodge, and J. L. Feder. 2014. The role of hybridization in a species invasion and extirpation of resident fauna: hybrid vigor and breakdown in the rusty crayfish, *Orconectes rusticus*. Journal of Crustacean Biology 34:157–164.

Armbruster, P., W. E. Bradshaw, and C. M. Holzapfel. 1997. Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. Evolution 51:451–458.

Arnold, M. L. 1992. Natural hybridization as an evolutionary process. Annual Review of Ecology and Systematics 23:237–261.

Arnold, M. L., and K. Kunte. 2017. Adaptive genetic exchange: a tangled history of admixture and evolutionary innovation. Trends in Ecology & Evolution 32:601–611.

Arnold, M. L., and N. H. Martin. 2009. Adaptation by introgression. Journal of Biology 8:82.

Avise, J. C., C. D. Ankney, and W. S. Nelson. 1990. Mitochondrial gene trees and the evolutionary relationship of mallard and black ducks. Evolution 44:1109–1119.

Baldassarre, G. A. 2014. Ducks, geese, and swans of North America. Volume 1. Johns Hopkins University Press, Baltimore, Maryland, USA.

Bandelt, H. J., P. Forster, and A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Barrett, J. 1973. Breeding behavior of captive mallards. Thesis, University of Minnesota, Minneapolis, USA.

Barton, N. H. 2001. The role of hybridization in evolution. Molecular Ecology 10:551–568.

Bellrose, F. C. 1980. Ducks, geese and swans of North America. Third edition. Stackpole, Harrisburg, Pennsylvania, USA.

Boecklen, W. J., and D. J. Howard. 1997. Genetic analysis of hybrid zones: numbers of markers and power of resolution. Ecology 78:2611–2616.

Bolstad, G. H., K. Hindar, G. Robertsen, B. Jonsson, H. Sægrov, O. H. Diserud, P. Fiske, A. J. Jensen, K. Urdal, and T. F. Næsje. 2017. Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. Nature Ecology and Evolution 1:0124.

Burns, J. T., K. M. Cheng, and F. McKinney. 1980. Forced copulation in captive mallards. I. Fertilization of eggs. Auk 97:875–879.

Bush, K. L., M. D. Vinsky, C. L. Aldridge, and C. A. Paszkowski. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus uropihasianus*). Conservation Genetics 6:867–870.

Caniglia, R., M. Galaverni, E. Velli, F. Mattucci, A. Canu, M. Apollonio, N. Mucci, M. Scandura, and E. Fabbri. 2020. A standardized approach to empirically define reliable assignment thresholds and appropriate management categories in deeply introgressed populations. Scientific Reports 10:1–14.

Cheng, K. M., J. T. Burns, and F. McKinney. 1982. Forced copulation in captive mallards (*Anas platyrhynchos*): II. temporal factors. Animal Behaviour 30:695–699.

Cheng, K. M., J. T. Burns, and F. McKinney. 1983. Forced copulation in captive mallards III. Sperm competition. Auk 100:302–310.

Clarke, A. L., B. Sæther, and E. Roskaft. 1997. Sex biases in avian dispersal: a reappraisal. Oikos 79:429–438.

Conroy, M. J., M. W. Miller, and J. E. Hines. 2002. Identification and synthetic modeling of factors affecting American black duck populations. Wildlife Monographs 50:1–64.

Cooke, F., C. MacInnes, and J. Prevett. 1975. Gene flow between breeding populations of lesser snow geese. Auk 92:493–510.

Coulon, A., J. Fitzpatrick, R. Bowman, B. Stith, C. Makarewich, L. Stenzler, and I. Lovette. 2008. Congruent population structure inferred from dispersal behaviour and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). Molecular Ecology 17:1685–1701.

Coulter, M. W., and W. R. Miller. 1968. Nesting biology of black ducks and mallards in northern New England. Vermont Fish and Game Department, Montpelier, USA.

DaCosta, J. M., and M. D. Sorenson. 2014. Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol. PLoS One 9:e106713.

Davis, E. S. 2002. Male reproductive tactics in the mallard, *Anas platyrhynchos*: social and hormonal mechanisms. Behavioral Ecology and Sociobiology 52:224–231.
Devers, P., and B. Collins. 2011. Conservation action plan for the American black duck. United States Department of the Interior, U.S. Fish and Wildlife Service, Division of Migratory Bird Management, Laurel, Maryland, USA.
Dobzhansky, T., and T. G. Dobzhansky. 1971. Genetics of the evolutionary process. Volume 139. Columbia University Press, New York, New York, USA.
Ellegren, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites-a new approach to studies of genetic relationships in birds. Auk 109:886–895.
Ellison, C. K., and R. S. Burton. 2008. Interpopulation hybrid breakdown maps to the mitochondrial genome. Evolution 62:631–638.
Fair, J., E. Paul, and J. Jones, editors. 2010. Guidelines to the use of wild birds in research. Ornithological Council, Washington, D.C., USA.
Ford, R. J., W. Selman, and S. S. Taylor. 2017. Hybridization between mottled ducks (*Anas fulvigula* maculosa) and mallards (*A. platyrhynchos*) in the western Gulf Coast region. Condor: Ornithological Applications 119:683–696.
Francis, C. M., J. R. Sauer, and J. R. Serie. 1998. Effect of restrictive harvest regulations on survival and recovery rates of American black ducks. Journal of Wildlife Management 62:1544–1557.
Francis, R. M. 2017. Pophelper: an R package and web app to analyze and visualize population structure. Molecular Ecology Resources 17:27–32.
Galloway, L. F., and C. B. Fenster. 1999. The effect of nuclear and cytoplasmic genes on fitness and local adaptation in an annual legume, *Chamaecrista fasciculata*. Evolution 53:1734–1743.
Grant, P. R., and B. R. Grant. 1992. Hybridization of bird species. Science 256:193–197.
Green, A. J. 1996. Analyses of globally threatened Anatidae in relation to threats, distribution, migration patterns, and habitat use. Conservation Biology 10:1435–1445.
Greenwood, P. J., and P. H. Harvey. 1982. The natal and breeding dispersal of birds. Annual Review of Ecology and Systematics 13:1–21.
Hall, M. C., and J. H. Willis. 2005. Transmission ratio distortion in intraspecific hybrids of *Mimulus guttatus*: implications for genomic divergence. Genetics 170:375–386.
Hans, J. B., A. Haubner, M. Arandjelovic, R. A. Bergl, T. Fünfstück, M. Gray, D. B. Morgan, M. M. Robbins, C. Sanz, and L. Vigilant. 2015. Characterization of MHC class II B polymorphism in multiple populations of wild gorillas using non-invasive samples and next-generation sequencing. American Journal of Primatology 77:1193–1206.
Hepp, G., J. M. Novak, K. Scribner, and P. Stangel. 1988. Genetic distance and hybridization of black ducks and mallards: a morph of a different color? Auk 105:804–807.
Heusmann, H. 1974. Mallard-black duck relationships in the northeast. Wildlife Society Bulletin 2:171–177.
Höglund, J., T. Johansson, A. Beintema, and H. Schekkerman. 2009. Phylogeography of the black-tailed godwit *Limosa limosa*: substructuring revealed by mtDNA control region sequences. Journal of Ornithology 150:45–53.
Hudson, R. R., M. Slatkin, and W. P. Maddison. 1992. Estimating of levels of gene flow from DNA sequence data. Genetics 13:583–589.
Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.
Janjua, S., J. L. Peters, B. Weckworth, F. I. Abbas, V. Bahn, O. Johansson, and T. P. Rooney. 2019. Improving our conservation genetic toolkit: ddRAD-seq for SNPs in snow leopards. Conservation Genetics Resources 12:257–261.
Johnsgard, P. A. 1960. Hybridization in the Anatidae and its taxonomic implications. Condor 62:25–33.
Johnson, K. P., and M. D. Sorenson. 1999. Phylogeny and biogeography of dabbling ducks (genus: *Anas*): a comparison of molecular and morphological evidence. Auk 116:792–805.
Jombart, T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.
Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.
Kimura, M., and T. Ohta. 1973. The age of a neutral mutant persisting in a finite population. Genetics 75:199–212.
Kirby, R. E., G. A. Sargeant, and D. Shutler. 2004. Haldane's rule and American black duck × mallard hybridization. Canadian Journal of Zoology 82:1827–1831.
Klett, A. T., H. F. Duebbert, C. A. Faanes, and K. F. Higgins. 1986. Techniques for studying nest success of ducks in upland habitats in the prairie pothole region. Resource Publication 158. U.S. Fish and Wildlife Service, Northern Prairie Wildlife Research Center, Jamestown, North Dakota, USA.
Kulikova, I. V., S. V. Drovetski, D. D. Gibson, R. J. Harrigan, S. Rohwer, M. D. Sorenson, K. Winker, Y. N. Zhuravlev, and K. G. McCracken. 2005. Phylogeography of the mallard (*Anas platyrhynchos*): hybridization, dispersal, and lineage sorting contribute to complex geographic structure. Auk 122:949–965.
Kulikova, I. V., Y. N. Zhuravlev, and K. G. McCracken. 2004. Asymmetric hybridization and sex-biased gene flow between eastern spot-billed ducks (*Anas zonorhyncha*) and mallards (*A. platyrhynchos*) in the Russian Far East. Auk 121:930–949.
Latta, R. G., K. M. Gardner, and A. D. Johansen-Morris. 2007. Hybridization, recombination, and the genetic basis of fitness variation across environments in *Avena barbata*. Genetica 129:167–177.
Lavretsky, P., J. M. Dacosta, B. E. Hernández-Baños, A. Engilis, Jr., M. D. Sorenson, and J. L. Peters. 2015. Speciation genomics and a role for the Z chromosome in the early stages of divergence between Mexican ducks and mallards. Molecular Ecology 24:5364–5378.
Lavretsky, P., J. M. DaCosta, M. D. Sorenson, K. G. McCracken, and J. L. Peters. 2019*a*. ddRAD-seq data reveal significant genome-wide population structure and divergent genomic regions that distinguish the mallard and close relatives in North America. Molecular Ecology 28:2594–2609.
Lavretsky, P., B. E. Hernández-Baños, and J. L. Peters. 2014. Rapid radiation and hybridization contribute to weak differentiation and hinder phylogenetic inferences in the New World mallard complex (*Anas* spp.). Auk: Ornithological Advances 131:524–538.
Lavretsky, P., T. Janzen, and K. G. McCracken. 2019*b*. Identifying hybrids and the genomics of hybridization: mallards & American black ducks of eastern North America. Ecology and Evolution 9:3470–3490.
Lavretsky, P., N. R. McInerney, J. E. Mohl, J. I. Brown, H. F. James, K. G. McCracken, and R. C. Fleischer. 2019*c*. Assessing changes in genomic divergence following a century of human mediated secondary contact among wild and captive-bred ducks. Molecular Ecology 29:578–595.
Lebigre, C., R. V. Alatalo, and H. Siitari. 2010. Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). Molecular Ecology 19:1929–1939.
Leipold, M., S. Tausch, M. Hirtreiter, P. Poschlod, and C. Reisch. 2020. Sampling for conservation genetics: how many loci and individuals are needed to determine the genetic diversity of plant populations using AFLP? Conservation Genetics Resources 12:99–108.
Livezey, B. C. 1991. A phylogenetic analysis and classification of recent dabbling ducks (Tribe Anatini) based on comparative morphology. Auk 108:471–507.
Longcore, J. R., D. G. McAuley, D. A. Clugston, C. M. Bunck, J. Giroux, C. Ouellet, G. R. Parker, P. Dupuis, D. B. Stotts, and J. R. Goldsberry. 2000. Survival of American black ducks radiomarked in Quebec, Nova Scotia, and Vermont. Journal of Wildlife Management 64:238–252.
Malinsky, M., E. Trucchi, D. Lawson, and D. Falush. 2018. RADpainter and fineRADstructure: population inference from RADseq data. Molecular Biology and Evolution 35:1284–1290.
Mallet, J. 2007. Hybrid speciation. Nature 446:279.
Mank, J. E., J. E. Carlson, and M. C. Brittingham. 2004. A century of hybridization: decreasing genetic distance between American black ducks and mallards. Conservation Genetics 5:395–403.
McFarlane, S. E., and J. M. Pemberton. 2019. Detecting the true extent of introgression during anthropogenic hybridization. Trends in Ecology and Evolution 34:315–326.
Mineau, P., F. McKinney, and S. R. Derrickson. 1983. Forced copulation in waterfowl. Behaviour 86:250–293.
Morin, P. A., J. Messier, and D. S. Woodruff. 1994. DNA extraction, amplification, and direct sequencing from hornbill feathers. Journal of The Science Society of Thailand 20:994.
Nazareno, A. G., J. B. Bemmels, C. W. Dick, and L. G. Lohmann. 2017. Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. Molecular Ecology Resources 17:1136–1147.

Nichols, J. D., H. H. Obrecht III, and J. E. Hines. 1987. Survival and band recovery rates of sympatric American black ducks and mallards. Journal of Wildlife Management 51:700–710.
O'Connell, K. A., K. P. Mulder, J. Maldonado, K. L. Currie, and D. M. Ferraro. 2019. Sampling related individuals within ponds biases estimates of population structure in a pond-breeding amphibian. Ecology and Evolution 9:3620–3636.
Parnell, J. F., and T. L. Quay. 1962. The populations, breeding biology, and environmental relations of the black duck, gadwall and blue-winged teal at Pea and Bodie Islands, North Carolina. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 16:53–67.
Pearce, J., R. Fields, and K. Scribner. 1997. Nest materials as a source of genetic data for avian ecological studies. Journal of Field Ornithology 68:471–481.
Perry, G. H., J. C. Marioni, P. Melsted, and Y. Gilad. 2010. Genomic-scale capture and sequencing of endogenous DNA from feces. Molecular Ecology 19:5332–5344.
Peters, J. L., P. Lavretsky, J. M. DaCosta, R. R. Bielefeld, J. C. Feddersen, and M. D. Sorenson. 2016. Population genomic data delineate conservation units in mottled ducks (*Anas fulvigula*). Biological Conservation 203:272–281.
Pfeifer, B., U. Wittelsbürger, S. E. Ramos-Onsins, and M. J. Lercher. 2014. PopGenome: an efficient Swiss army knife for population genomic analyses in R. Molecular Biology and Evolution 31:1929–1936.
Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. De Bakker, and M. J. Daly. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81:559–575.
R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Randi, E. 2008. Detecting hybridization between wild species and their domesticated relatives. Molecular Ecology 17:285–293.
Reed, A. 1975. Reproductive output of black ducks in the St. Lawrence estuary. Journal of Wildlife Management 39:243–255.
Rhymer, J. M. 2006. Extinction by hybridization and introgression in anatine ducks. Acta Zoological Sinica 52:583–585.
Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27:83–109.
Ringelman, J. K., J. R. Longcore, and R. B. Owen, Jr. 1982. Nest and brood attentiveness in female black ducks. Condor 84:110–116.
Ringelman, K. M., and C. K. Williams. 2018. The American black duck: three decades of science-based adaptive management. Case Studies in the Environment 2:1–8.
Rohwer, F. C., and M. G. Anderson. 1988. Female-biased philopatry, monogamy and the timing of pair formation in waterfowl. Current Ornithology 5:187–221.
Scherer, S., and T. Hilsberg. 1982. Hybridisierung und Verwandtschaftsgrade innerhalb der Anatidae—eine systematische und evolutions theoretische Betrachtung. Journal of Ornithology 123:357–380.
Schmaltz, G., C. M. Somers, P. Sharma, and J. S. Quinn. 2006. Non-destructive sampling of maternal DNA from the external shell of bird eggs. Conservation Genetics 7:543–549.
Seehausen, O. 2006. Conservation: losing biodiversity by reverse speciation. Current Biology 16:R334–R337.
Seehausen, O., G. Takimoto, D. Roy, and J. Jokela. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. Molecular Ecology 17:30–44.
Seymour, N. R. 1990. Forced copulation in sympatric American black ducks and mallards in Nova Scotia. Canadian Journal of Zoology 68:1691–1696.
Seymour, N. R. 1991. Philopatry in male and female American black ducks. Condor 93:189–191.
Simberloff, D. 1996. Hybridization between native and introduced wildlife species: importance for conservation. Wildlife Biology 2:143–150.
Sorenson, M. D., J. C. Ast, D. E. Dimcheff, T. Yuri, and D. P. Mindell. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Molecular Phylogenetics and Evolution 12:105–114.
Sorenson, M. D., and R. C. Fleischer. 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. Proceedings of the National Academy of Sciences 93: 15239–15243.
Soutiere, E. C. 1986. Hand-reared mallard releases on 3 private farms in Maryland. Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies 40:438–445.
Stelkens, R. B., C. Schmid, and O. Seehausen. 2015. Hybrid breakdown in cichlid fish. PLoS One 10:e0127207.
Strausberger, B., and M. V. Ashley. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos and offspring. Conservation Genetics 2:385–390.
Taberlet, P., and J. Bouvet. 1991. A single plucked feather as a source of DNA for bird genetic studies. Auk 108:959–960.
Taberlet, P., and L. Fumagalli. 1996. Owl pellets as a source of DNA for genetic studies of small mammals. Molecular Ecology 5:301–305.
Taberlet, P., and L. P. Waits. 1998. Non-invasive genetic sampling. Trends in Ecology & Evolution 1:26–27.
Taberlet, P., L. P. Waits, and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. Trends in Ecology & Evolution 14:323–327.
Trimbos, K. B., J. Broekman, R. Kentie, C. J. Musters, and G. R. de Snoo. 2009. Using eggshell membranes as a DNA source for population genetic research. Journal of Ornithology 150:915–920.
Trimbos, K. B., C. Doorenweerd, K. Kraaijeveld, C. J. Musters, N. M. Groen, P. de Knijff, T. Piersma, and G. R. de Snoo. 2014. Patterns in nuclear and mitochondrial DNA reveal historical and recent isolation in the black-tailed godwit (*Limosa limosa*). PLOS ONE 9:e83949.
U.S. Fish and Wildlife Service. 2013. Review of captive-reared mallard regulations on shooting preserves. Final Report. Assistant Director of Migratory Birds, Washington, D.C., USA.
Vallejo-Marín, M., and S. J. Hiscock. 2016. Hybridization and hybrid speciation under global change. New Phytologist 211:1170–1187.
Van Dijk, E. L., Y. Jaszczyszyn, and C. Thermes. 2014. Library preparation methods for next-generation sequencing: tone down the bias. Experimental Cell Research 322:12–20.
Vilà, M., E. Weber, and C. M. Antonio. 2000. Conservation implications of invasion by plant hybridization. Biological Invasions 2:207–217.
Wakeley, J. 1996. The variance of pairwise nucleotide differences in two populations with migration. Theoretical Population Biology 49:39–57.
Wang, J. 2018. Effects of sampling close relatives on some elementary population genetics analyses. Molecular Ecology Resources 18:41–54.
Wang, J., and A. Caballero. 1999. Developments in predicting the effective size of subdivided populations. Heredity 82:212–226.
Wells, C. P., P. Lavretsky, M. D. Sorenson, J. L. Peters, J. M. DaCosta, S. Turnbull, K. J. Uyehara, C. P. Malachowski, B. D. Dugger, and J. M. Eadie. 2019. Persistence of an endangered native duck, feral mallards, and multiple hybrid swarms across the main Hawaiian Islands. Molecular Ecology 28:5203–5216.
Wong, P. B., E. O. Wiley, W. E. Johnson, O. A. Ryder, S. J. O'Brien, D. Haussler, K. Koepfli, M. L. Houck, P. Perelman, and G. Mastromonaco. 2012. Tissue sampling methods and standards for vertebrate genomics. Gigascience 1:8.
Zhou, H., D. Alexander, and K. Lange. 2011. A quasi-Newton acceleration for high-dimensional optimization algorithms. Statistics and Computing 21:261–273.
Zhuravlev, Y. N., and I. V. Kulikova. 2014. Waterfowl population structure: phylogeographic inference. Achievements in the Life Sciences 8:123–127.
Zimpfer, N. L. 2004. Estimating movement and production rates in American black ducks. Thesis, University of Georgia, Athens, USA.

Associate Editor: Jacob Straub.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.