HYBRIDIZATION AND POPULATION STRUCTURE OF WESTERN GULF COAST MOTTLED DUCKS

A Thesis

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ABSTRACT

Rates of hybridization between species that do not normally interbreed have increased due to human impacts on natural environments, such as habitat alteration or introductions of non-native species. Human-induced hybridization can be detrimental to wildlife and contribute to species extinctions. In Florida, feral Mallards (*Anas platyrhynchos*) hybridize with endemic Mottled Ducks (*A. fulvigula*) at rates close to 9%. However, levels of hybridization between these two species have not been extensively examined in the western Gulf Coast (from Alabama to northern Mexico) despite the potential impact on the Mottled Duck lineage. In this study, I examined the degree of hybridization between Mottled Ducks and Mallards in the western Gulf Coast. In addition, I validated a key developed in Florida to distinguish Mottled Ducks from Mallards and their hybrids for western Gulf Coast Mottled Ducks. Lastly, I examined the genetic structure of Mottled Duck populations and estimated gene flow by determining the number of migrants between regions of Mottled Ducks across the western Gulf Coast. I used 36 microsatellite loci to genotype 405 ducks including putative Mottled Ducks, Mallards, and hybrids. Overall, genetic analyses revealed very low rates of hybridization (5.4%) in the western Gulf Coast. The key to distinguish Florida Mottled Ducks from Mallards and their hybrids proved highly effective (97%) for the western Gulf Coast population. Finally, multiple analyses indicated that Mottled Ducks are a single genetic population across the western Gulf Coast, which may be primarily due to dispersal of juvenile ducks. Currently, hybridization with Mallards is not a threat to western Gulf Coast Mottled Ducks; however, Mottled Duck hybridization should be monitored in the future to ensure that hybridization rates do not increase.

CHAPTER 1. INTRODUCTION

The Mottled Duck (*Anas fulvigula*) is a non-migratory, coastal dabbling duck species with populations in Florida and along the western Gulf Coast from Alabama to northern Mexico. Currently, Mottled Ducks are considered a species of conservation concern in Louisiana (Lester et al. 2005), primarily due to a declining population caused by alterations to marsh habitat. Loss of marsh habitat is particularly threatening to western Gulf Coast Mottled Ducks because, unlike other North America waterfowl species, Mottled Ducks meet all life-history stages usually within 30 km of the coastline (Stutzenbaker 1988).

A new and critical threat to Mottled Ducks is hybridization. Rates of hybridization between species that do not normally interbreed have increased due to human impacts on natural environments, such as deforestation for urban development or introductions of non-native species. Human induced hybridization may be detrimental to wildlife by increasing the loss of genetically distinct lineages through introgressive hybridization. Recently, Mottled Duck breeding grounds have been increasingly invaded by Mallards that do not migrate north (Wilson 2007). Mottled Ducks and Mallards that congregate, particularly when pairing, is a concern because interbreeding between Mallards and all North American species within the Mallard complex (Mexican Duck, *A. diazi*, American Black Duck, *A. rubripes*, and Mottled Duck, *A. fulvigula*) results in viable and fertile offspring (Brodsky and Weatherhead 1984; Williams et al. 2005a). Currently, a major emerging concern in the western Gulf Coast region of the United States is hybridization between Mallards and endemic Mottled Ducks. In Florida, Mottled Duck hybridization has become such a concern that the Florida Fish and Wildlife Conservation Commission passed legislation in the early 2000s prohibiting the possession and release of captive Mallards. In the western Gulf Coast, levels and areas of hybridization are unknown;

therefore, an investigation of interbreeding between Mottled Ducks and Mallards is needed to reveal the extent of the problem.

In addition to the uncertainty regarding hybridization between Mottled Ducks and Mallards, the finer-scale genetic structuring of the western Gulf Coast Mottled Duck population is unknown and would be useful for conservation managers in order to manage them properly. Mottled Ducks occur in two distinct subspecies as shown by band-recovery and genetic data: one subspecies occurs in peninsular Florida (*A. fulvigula fulvigula*), and the other is a resident of the Gulf Coast from Alabama to northeastern Mexico (*A. f. maculosa*; McCracken et al. 2001, Williams et al. 2005b; Wilson 2007; Baldassarre 2014). Mottled Ducks may also show genetic structuring on a finer scale in the western Gulf Coast due to restricted bird movements or barriers to gene flow. For example, restricted birds movements (limited home ranges) or low dispersal (migrants) could prevent gene flow across the Gulf Coast.

In this study, I used population genetics to investigate hybridization and the geographic structure of genetic variation in western Gulf Coast Mottled Ducks. In chapter 2, I determined the degree of hybridization between Mottled Ducks and Mallards. In chapter 3, I applied an experimental key developed to distinguish Florida Mottled Ducks from Mallards and their hybrids to Mottled Ducks for validation in the western Gulf Coast. In chapter 4, I determined the number genetic Mottled Duck populations and estimated migration rates between geographic regions of Mottled Ducks across the western Gulf Coast. Data on hybridization rates, along with the validation of the key to distinguish Mottled Ducks from Mallards and their hybrids, will allow managers to cull hybrids and Mallards that remain on western Gulf Coast Mottled Duck breeding grounds to prevent future hybridization. Similarly, data on genetic structure should help managers make important conservation decisions in order to protect any genetically distinct

populations. Ultimately, these management decisions will increase the probability that Mottled Duck genetic variation is protected.

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CHAPTER 2. HYBRIDIZATION BETWEEN MALLARDS AND WESTERN GULF COAST MOTTLED DUCKS

Hybridization is the interbreeding of individuals from genetically distinct populations, regardless of their taxonomic status (Grant and Grant 1992; Allendorf et al. 2001; Allendorf and Luikart 2007). Natural hybridization plays an important role in the evolution of plant and animal species because organisms can acquire favorable adaptations and novel gene combinations (Seehausen 2004; Allendorf and Luikart 2007). However, when extensive hybridization has an anthropogenic cause, it can threaten the genetic integrity of distinct species. Rates of hybridization between species that do not normally interbreed have increased due to human impacts on natural environments, such as deforestation for urban development or introductions of non-native species, which can bring once allopatric species into contact. Human induced hybridization may be detrimental to wildlife and can contribute to species extinctions in two ways; first, population growth rates may be reduced when sterile or partially sterile hybrids are produced (Allendorf and Luikart 2007). For instance, when native female European Mink (*Mustela lutreola*) hybridize with male introduced North American Mink (*Neovision vison*), embryos are aborted and reproductive opportunities are wasted, which has contributed to the decline of the European species (Rozhnov 1993). Second, when hybrids are fertile, hybridization may lead to the loss of genetically distinct lineages through introgressive hybridization. Introgression is the incorporation of genes from one species into another and occurs when viable hybrids backcross with individuals from parental populations (Allendorf et al. 2001). For example, nearly a third of phenotypically representative Golden-winged Warbler (*Vermivora chrysoptera*) genomes have been introgressed with Blue-winged Warbler (*V. cyanoptera*) genes as shown by AFLP (amplified fragment length polymorphism) data, suggesting that there are not as many genetically representative *chrysoptera* individuals as previously thought (Vallender et

al. 2007). When hybrids continue to backcross to parental populations, a complete admixture of genomes (hybrid swarm) is formed, leading to genomic extinction of parental lineages (Allendorf and Luikart 2007).

Hybridization may occur for various reasons, including introductions of species, mistakes in mate recognition, and/or a scarcity of conspecifics. Hybridization often occurs when new exotic species are introduced into the range of related species. For instance, the introduction of domestic reindeer (*Rangifer tarandus tarandus*) into Alaska has resulted in hybridization with native caribou (*R. t. granti*) on Alaska's North Slope (Mager et al. 2013). Similarly, populations of native westslope cutthroat trout (*Oncorhychus clarki lewisi*) hybridize with rainbow trout (*O. mykiss*) that were introduced to the upper Columbia River system in Idaho and British Columbia (Rubidge and Taylor 2004). Mistakes in mate recognition can result in hybridization between two species (Randler 2002). Assortative mating may be responsible for interspecific mating between native and introduced species when they have similar body sizes (Crespi 1989), plumage or coloration, and possibly, breeding displays. Lastly, hybridization could transpire when individuals choose heterospecific mates because conspecifics are absent or already paired (Randler 2008). Individuals might mate with heterospecifics in order to produce any viable offspring rather than remain unpaired or abandon breeding altogether (Randler 2008).

Hybridization between avian species is relatively common, but it is most prevalent in Anseriformes (ducks, swans, and geese), which show the highest tendency to hybridize among birds (Grant and Grant 1992; McCarthy 2006). One anseriform that frequently hybridizes, especially when introduced to new areas, is the Mallard (*Anas platyrhynchos*). Mallards, native to and distributed throughout most of the Holarctic, are the most numerous and widespread waterfowl species (Kulikova et al. 2005). Mallards hybridize with at least 23 other *Anas* species

globally (Marchant and Higgins 1990), in both their native range as well as when introduced to new regions. For example, the introduction of Mallards into New Zealand has almost certainly caused the extinction of native Grey Ducks (*A. superciliosa superciliosa*) due to introgressive hybridization (Rhymer et al. 1994). This has become a significant conservation problem because interbreeding between Mallards and all North American species within the Mallard complex (Mexican Duck, *A. diazi*, American Black Duck, *A. rubripes*, and Mottled Duck, *A. fulvigula*) results in viable and fertile offspring (Brodsky and Weatherhead 1984). Currently, an emerging concern in the western Gulf Coast region of the United States is hybridization between Mallards and endemic Mottled Ducks.

The Mottled Duck is a non-migratory, harvested dabbling duck with native populations in Florida (*A. fulvigula fulvigula*) and the western Gulf Coast (*A. fulvigula maculosa*). Mottled Ducks are the most abundant breeding waterfowl species in the coastal marshes of Louisiana and Texas, meeting all life history requirements in non-tidal, fresh to brackish ponds of coastal marshes, emergent wetlands, flooded rice fields, and tidal wetlands of major river deltas (e.g., Mississippi and Atchafalaya river deltas; Stutzenbaker 1988; Bielefeld et al. 2010). Recently in Florida, Mottled Duck breeding grounds have been increasingly invaded by Mallards, primarily due to habitat modifications and releases of farm-reared Mallards (Williams et al. 2005a). It has been estimated that roughly 12,000 Mallards have been released annually throughout Florida since the early 1990s (Bielefeld et al. 2010). Accordingly, hybridization is now an emerging conservation concern of managers of western Gulf Coast Mottled Ducks. The problem of hybridization between Mottled Ducks and Mallards may be exacerbated by a reduction in Mottled Duck habitat due to wetland drainage, degradation of coastal marshes, and urban development (Bielefeld et al. 2010). Degradation of habitat could force Mottled Duck

populations to share breeding habitat with Mallards and further increase the probability of hybridization, especially since both share nearly identical courtship displays (Moorman and Gray 1994). Thus, contact between Mottled Ducks and Mallards is concerning because interbreeding could produce hybrids that are as fit or more fit than Mottled Ducks in coastal habitats (a phenomenon known as heterosis; Allendorf et al. 2007).

Hybridization between Mottled Ducks and Mallards in Florida is ~9.3%, where Mallards (assumed to be captive-reared) remain on Mottled Duck breeding areas during the breeding season (Williams et al. 2005a). Further, 10.9% of Mottled Ducks sampled were found to be hybrids, whereas only 3.4% of sampled Mallards were deemed hybrids (Williams et al. 2005a). This implies asymmetric introgression, with Mallard genes being incorporated into the Mottled Duck genome at a higher rate than Mottled Duck genes transfer into the Mallard genome. Asymmetric introgression might occur because hybrids assimilate into and breed within Mottled Duck populations (Williams et al. 2005a). If hybridization continues, the unique Mottled Duck genome could disappear in Florida. Furthermore, it appears that genomic extinction of Mottled Ducks has occurred in South Carolina, where Mottled Ducks translocated from Texas, Louisiana, and Florida from 1975 – 1983 have interbred with Mallards and now all appear to be hybrids (Williams et al. 2005a). Mottled Duck hybridization has become such a concern in Florida that the Florida Fish and Wildlife Conservation Commission passed legislation in the early 2000s prohibiting the possession and release of captive Mallards.

Hybridization in western Gulf Coast Mottled Ducks has been briefly studied with a limited number of loci and samples (Peters et al. 2014). Peters et al. (2014) used six loci and 78 samples (40 putative Mottled Ducks and 38 putative hybrids) to genetically distinguish Mottled Ducks and hybrids using genetic probability assignments (≥ 80% ancestry assigned individuals

to a species whereas as < 80% ancestry assigned them as a hybrid). The majority of putative hybrids were actually Mottled Ducks, while only a few were hybrids and one was a Mallard (Peters et al. 2014). Further, 95% of putative Mottled Ducks were genetically assigned as Mottled Ducks and 5% were hybrids. Despite only using six loci, the authors conclude that they are sufficient for distinguishing Mottled Ducks from Mallards but provide low power for dependably detecting hybrids. Identifying hybrids requires numerous loci, and for closely related species ($0.1 < F_{ST} < 0.2$), at least 12 – 24 polymorphic microsatellite loci are needed to recognize F1 individuals as hybrids (Vähä and Primmer 2006). Therefore, a more rigorous investigation of hybridization between Mottled Ducks and Mallards using more genetic markers is needed in the western Gulf Coast to determine whether introgression is a serious problem. Accordingly, this is an area of research that has been identified as a priority action by the Gulf Coast Joint Venture Mottled Duck Conservation Plan (Wilson 2007).

In this chapter, I estimated the degree of hybridization between Mottled Ducks and Mallards in the western Gulf Coast.

2.1 METHODS

2.1.1 Sampling

Mottled Ducks were caught by hand from an airboat at night with the aid of spotlights and an experienced airboat operator during the summer molt (June – August) along coastal Louisiana from 2011 – 2014 in conjunction with Louisiana Department of Wildlife and Fisheries banding operations. Upon capture, blood was drawn from each duck by brachial vein puncture (approximately 50 – 100 µl) and stored in Queen's lysis buffer (Seutin et al. 1991), and ducks were released safely afterwards. In order to sample the entire range of western Gulf Coast

Mottled Ducks, additional samples were collected via donations from hunters and by wildlife biologists from 2009 – 2014. In Louisiana, hunters donated ducks from Caernarvon (2012 and 2013) and the Louisiana Department of Wildlife and Fisheries (LDWF) collected samples from Cameron Prairie National Wildlife Refuge (2014). In Texas, the Texas Parks and Wildlife Department collected samples from Justin Hurst Wildlife Management Area, J.D. Murphree Wildlife Management Area, Guadalupe Delta Wildlife Management Area, and Mad Island Wildlife Management Area (2012 – 2014). In Alabama, the Alabama Department of Conservation and Natural Resources collected samples from Mobile Bay and the Mobile-Tensaw Delta (November 2013). Mallard tissue samples were obtained from hunter harvested ducks in 2014 across mid-northern Louisiana (collected by LDWF) and collections at Big Burns Marsh (acquired by LDWF with permission from the landowner, Miami Corporation, to conduct research and banding operations) and Cameron Prairie National Wildlife Refuge (2014). Additionally, Mallard wings from hunter harvested ducks were collected in Mississippi (acquired by Mississippi Department of Wildlife, Fisheries, and Parks biologists, January 2014) and in Alabama at Mobile Bay and the Mobile-Tensaw Delta. Sampling information is summarized in Figure 2.1 and Table 2.1. Ducks were classified to species by the collector (i.e. we initially assumed the species assignment upon collection was correct).

2.1.2 Microsatellite Genotyping and mtDNA Sequencing

DNA was extracted from putative Mottled ducks (n = 319), Mallards (n = 76), and hybrids (n = 10) using Qiagen DNeasy Kits (Qiagen Inc., Valencia, CA) and screened for amplification and polymorphism with 36 microsatellite loci developed for Mottled Ducks (Seyoum et al. 2012, Appendix 1). All forward primers were labeled at the 5’ end with a M13 -

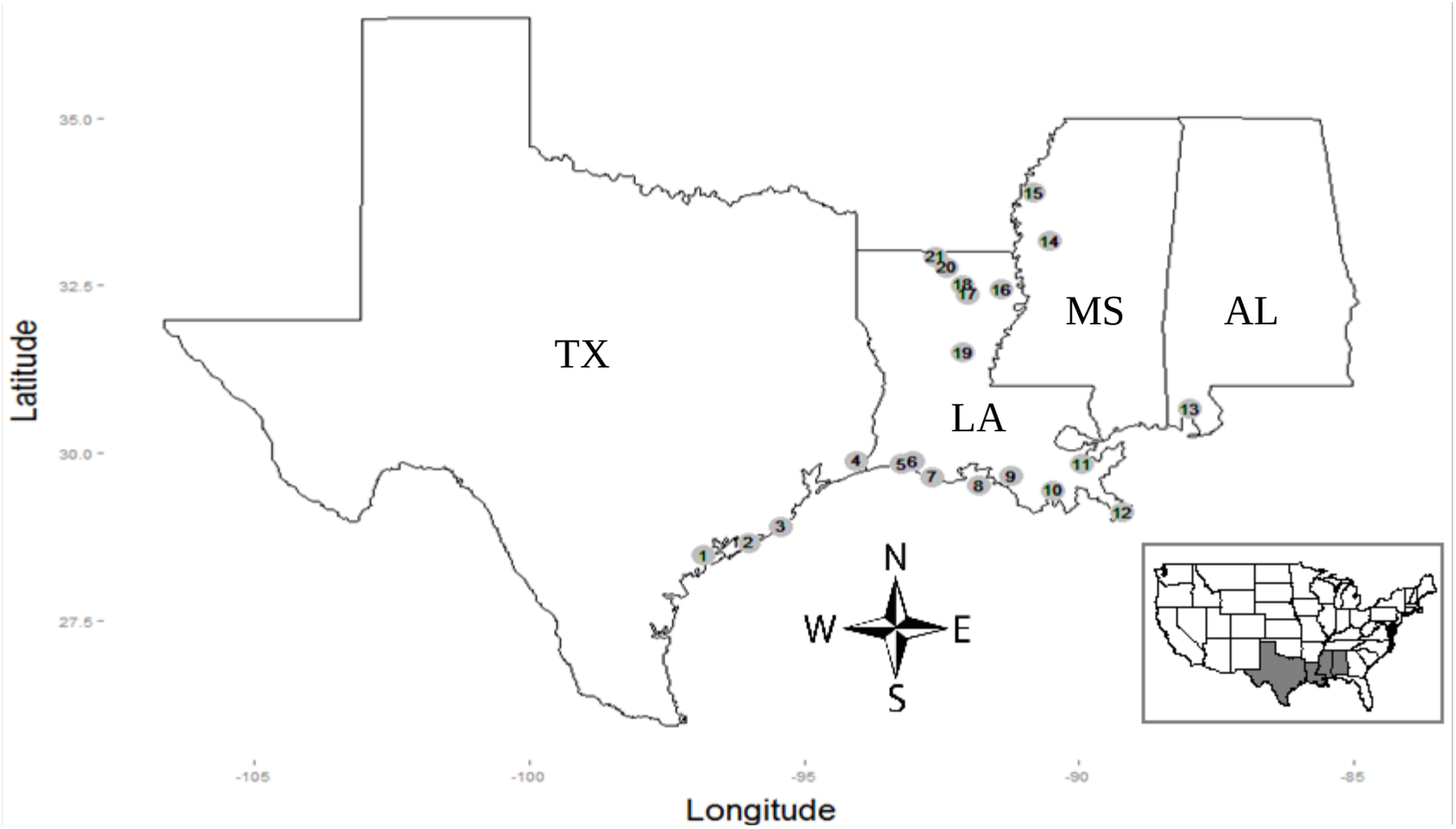

Figure 2.1 Distribution of sample collection sites for Mottled Ducks, Mallards, and Hybrids. Sampling location abbreviations are as follows: Guadalupe Delta WMA, TX (1), Mad Island WMA, TX (2), Justin Hurst WMA, TX (3), J.D. Murphree WMA, TX (4), Cameron-Prairie NWR, LA (5), Big Burns Marsh, LA (6), Rockefeller SWR, LA (7), Marsh Island SWR, LA (8), Atchafalaya Delta WMA, LA (9), Point aux Chenes WMA, LA (10), Caernarvon, LA (11), Pass-a-Loutre WMA, LA (12), Mobile-Tensaw Delta, AL (13), Humphreys County, MS (14), Bolivar County, MS (15), Waverly, LA (16), Ouachita WMA, LA (17), Monroe, LA (18), Catahoula Lake, LA (19), Farmerville, LA (20), and Spearsville, LA (21).

Table 2.1 Species, geographic location, number of individuals sampled, and source of genetic material for 405 ducks used in this study.

Species	Geographic locality	No. Samples	Source of Genetic Material
Mottled Duck (*Anas f. maculosa*)	Atchafalaya Delta WMA, *St. Mary Parish, Louisiana*	8	Blood
	Big Burns Marsh, *Cameron Parish, Louisiana*	17	Blood
	Caernarvon, *St. Bernard Parish, Louisiana*	2	Breast muscle
	Cameron-Prairie NWR, *Cameron Parish, Louisiana*	10	Blood & Breast muscle
	J.D. Murphree WMA, *Jefferson County, Texas*	31	Breast muscle
	Justin Hurst WMA, *Brazoria County, Texas*	75	Blood & Breast muscle
	Mad Island WMA, *Matagorda County, Texas*	61	Breast muscle
	Marsh Island State Wildlife Refuge, *Iberia Parish, Louisiana*	29	Blood
	Mobile-Tensaw Delta, *Baldwin & Mobile Counties, Alabama*	5	Breast muscle
	Pass-A-Loutre State WMA, *Plaquemines Parish, Louisiana*	8	Blood
	Point aux Chenes WMA, *Lafourche & Terrebonne Parishes, Louisiana*	27	Blood
	Rockefeller Wildlife Refuge, *Cameron & Vermillion Parishes, Louisiana*	34	Blood
Mallard (*Anas platyrhynchos*)	Big Burns Marsh, *Cameron Parish, Louisiana*	5	Blood & Breast muscle
	Bolivar County, *Mississippi*	25	Wing muscle
	Caernarvon, *St. Bernard Parish, Louisiana*	1	Breast muscle
	Catahoula Lake, *La Salle & Rapides Parishes, Louisiana*	1	Wing muscle
	Farmerville, *Union Parish, Louisiana*	1	Wing muscle
	Humphreys County, *Mississippi*	22	Wing muscle
	J.D. Murphree WMA, *Jefferson County, Texas*	1	Breast muscle
	Justin Hurst WMA, *Brazoria County, Texas*	1	Breast muscle
	Mobile-Tensaw Delta, *Baldwin & Mobile Counties, Alabama*	2	Wing muscle
	Monroe, *Ouachita Parish, Louisiana*	5	Wing muscle
	Ouachita WMA, *Ouachita Parish, Louisiana*	6	Wing muscle
	Spearsville, *Union Parish, Louisiana*	3	Wing muscle
	Waverly, *Madison Parish, Louisiana*	3	Wing muscle

(Table 2.1 continued)

Species	Geographic locality	No. Samples	Source of Genetic Material
Hybrid	Atchafalaya Delta WMA, *St. Mary Parish, Louisiana*	2	Blood & Breast muscle
	Cameron Prairie NWR, *Cameron Parish, Louisiana*	1	Breast muscle
	Guadalupe Delta WMA, *Calhoun County, Texas*	1	Breast muscle
	Humphreys County, *Mississippi*	3	Wing muscle
	J.D. Murphree WMA, *Jefferson County, Texas*	1	Breast muscle
	Justin Hurst WMA, *Brazoria County, Texas*	3	Blood & Breast muscle
	Mad Island WMA, *Matagorda County, Texas*	3	Breast muscle
	Marsh Island State Wildlife Refuge, *Iberia Parish, Louisiana*	1	Blood
	Ouachita WMA, *Ouachita Parish, Louisiana*	1	Wing muscle
	Pass-A-Loutre State WMA, *Plaquemines Parish, Louisiana*	3	Blood
	Rockefeller Wildlife Refuge, *Cameron & Vermillion Parishes, Louisiana*	2	Blood
	Waverly, *Madison Parish, Louisiana*	1	Wing muscle

tail (5'-CACGACGTTGTAAAACGAC-3') to allow detection of alleles. PCR amplifications contained 20 ng DNA, 1X standard *Taq* (Mg-free) reaction buffer (New England BioLabs Inc., Ipswich, MA), 0.8 mM dNTPs (Qiagen Inc., Valencia, CA), 0.2 µl v/v 100% dimethyl sulfoxide (DMSO), 0.5 units *Taq* polymerase (New England BioLabs Inc., Ipswich, MA), 0.5 – 1 µM of each primer, 0.75 – 1.5 mM $MgCl_2$ (New England BioLabs Inc., Ipswich, MA), 0.2 – 0.8 M betaine, 0.1 – 0.3 µM 5' fluorescently labeled M13 forward primer (6FAM, NED, PET, or VIC; Applied Biosystems), and nanopure water for a final reaction volume of 10 µL (Appendix1). Reactions were ran on Eppendorf Mastercycler proS and BioRad MyCycler thermal cyclers with the following conditions: 2 minutes at 95°C for initial denaturation, followed by 35 – 45 cycles of 30 seconds at 94°C, 30 seconds at the annealing temperature (Appendix 1), and 30 seconds at 72°C for elongation, ending with a final elongation step of 72°C for 5 minutes.

Initially, amplification was optimized using a LI-COR 4200 GENE READIR DNA Analyzer (LI-COR Inc., Lincoln, NE). Alleles sizes were determined using appropriately labeled external size standards (IRDye700 or IRDye800, 50 – 350 bp sizing standard, LI-COR Inc., Lincoln, NE) and estimated using Saga version 3.2 (LI-COR Inc., Lincoln, NE). Following optimization, all samples were sent to the Yale DNA Analysis Facility on Science Hill (New Haven, CT) for DNA fragment analysis using an Applied Biosystems 3730 DNA genetic analyzer. Allele sizes were determined using LIZ-500 size standards added by the genotyping facility. A subset of samples was used on all fragment analyses to ensure consistent scoring of alleles. Genotypes were assigned using GENEMARKER version 2.4 (Soft Genetics, LLC., State College, PA).

Mitochondrial genes cytochrome b (*Cyt b*) and NADH dehydrogenase subunit 2 (*ND2*) were sequenced for hybrids (n = 22), western Gulf Coast Mottled Ducks (n = 15), and Mallards

(n = 11) using previously published primers (Appendix 2; Desjardins and Morais 1990; Johnson and Sorenson 1998; Donne-Goussé et al. 2002). PCR amplifications contained 50 ng DNA, 1X standard *Taq* (Mg-free) reaction buffer (New England BioLabs Inc., Ipswich, MA), 0.8 mM dNTPs (Qiagen Inc., Valencia, CA), 1.5 mM $MgCl_2$ (New England BioLabs Inc., Ipswich, MA), 0.8 units *Taq* polymerase (New England BioLabs Inc., Ipswich, MA), 1 µM of each primer, and nanopure water for a final reaction volume of 25 µL. Reactions were carried out on an Eppendorf Mastercycler proS thermal cycler with the following conditions: 5 minutes at 94°C for initial denaturation, followed by 40 cycles of 1 minute at 94°C, 1 minute at 57°C, and 1 minute at 72°C for elongation, ending with a final elongation step of 72°C for 10 minutes. Following amplification, samples were sent to Beckman Coulter Genomics (Danvers, MA) for Sanger sequencing.

2.2 STATISTICAL ANALYSES

2.2.1 Genetic Diversity

Measures of genetic diversity were estimated separately by species after removing ducks inferred to be hybrids based on genetic mixture analysis. Genetic diversity was measured in each species as allelic richness (AR), and observed (H_O) and expected heterozygosities (H_E) in program R using the hierfstat package (Goudet 2005). Exact tests for departures from Hardy-Weinberg expectations for each locus and linkage disequilibrium for each locus pair in each population were calculated in GENEPOP version 4.3 (Rousset 2008). Significance levels of multiple comparisons were corrected using sequential Bonferroni adjustments (Rice 1989) to maintain an overall experiment-wise error rate of $\alpha = 0.05$. Genetic differentiation between Mottled Ducks and Mallards was determined using F_{ST} and R_{ST} estimates in GENEPOP version

4.3 (Rousset 2008), where significant estimates were based on 95% confidence intervals and those bracketing zero were not significant. Additionally, differentiation was examined in a two-dimensional Principal Component Analysis (PCA) performed in R using the hierfstat package (Goudet 2005) plotting each individual using their respective microsatellite allele composition without prior species designation.

2.2.2 Genetic Mixture

Hybridization between Mottled Ducks and Mallards was inferred using the program STRUCTURE version 2.3.4 (Pritchard et al. 2000). STRUCTURE uses multi-locus genotype data and Bayesian clustering analyses to identify distinct populations, assign individuals to populations, and identify admixed individuals. In STRUCTURE, the user selects the number of populations (*K*) for each model, where each *K* is characterized by allele frequencies at each locus (Pritchard et al. 2000). This approach has been used to successfully identify hybrids in Florida (Williams et al. 2005a), and does not require prior identification of Mottled Ducks or Mallards.

Models ranging from a single-population model to a four-population model ($K = 1 – 4$) were tested using 10 replications for each model, a burn-in of 200,000 steps, followed by 1,000,000 Markov-Chain Monte Carlo iterations (MCMC). The admixture ancestry model and correlated allele frequencies were assumed among populations. To determine the most likely number of clusters (*K*) in the overall sample, output from STRUCTURE was used in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012) which evaluates the likelihood of each model and selects the best *K* using the Evanno method (Evanno et al. 2005). I used the quantified proportion of each individual's ancestry (*q*) to assign individuals to a species or as a hybrid. Individuals with ≥ 90% of their ancestry assigned to either the Mottled Duck or Mallard cluster

were considered to be a member of the species, whereas individuals with < 90% were considered to be hybrids. Finally, output from STRUCTURE was used in the program CLUMPAK (Kopelman et al. 2015) to create bar graphs for individuals according to their ancestry proportions (*q*) by population. Estimates provided in the results section are means ± standard error.

2.2.3 Hybrid Parentage

Mitochondrial DNA (mtDNA) sequences were used to assess hybrid maternal parentage. Since mtDNA is only inherited from mothers, all hybrid offspring will have maternal DNA. Therefore, we can examine mating patterns producing hybrids; for example, mothers of hybrids may tend to be Mottled Ducks. Sequences from mtDNA were corrected and trimmed in SEQUENCER 5.0 (Gene Codes Corp., Ann Arbor, MI) and visually inspected to determine the number of haplotypes for Mottled Ducks, Mallards, and hybrids to establish the percentage of each haplotype that occurs in each species.

2.3 RESULTS

2.3.1 Genetic Diversity

Mean allelic richness for Mottled Ducks was 10.093 ± 1.039, while mean observed and expected heterozygosities were 0.5609 ± 0.0383 and 0.6620 ± 0.0348, respectively (Table 2.2). Mean allelic richness for Mallards was 10.346 ± 0.937, while mean observed and expected heterozygosities were 0.5845 ± 0.0341 and 0.7086 ± 0.0350, respectively. Mottled Ducks showed linkage disequilibrium for 4.6% (29 out of 630) of Bonferroni-corrected pairwise comparisons among loci, whereas Mallards showed linkage disequilibrium for 0.2% (1 out of 630) of pairwise comparisons among loci.

Table 2.2 Genetic diversity measures for Mottled Ducks (*Anas f. maculosa*) and Mallards (*A. platyrhynchos*) including allelic richness (AR) and observed (H_O) and expected (H_E) heterozygosity (36 microsatellite loci). Bold indicates departure from Hardy-Weinberg equilibrium expectations ($P < 0.05$ after sequential Bonferroni correction).

	Anas fulvigula (*n* = 307)			*Anas platyrhynchos* (*n* = 76)		
Locus	AR	H_O	H_E	AR	H_O	H_E
Aful04	4.000	0.4226	0.5116	3.987	0.2361	0.2150
Aful05	14.604	0.8100	0.8300	11.925	0.7606	0.8880
Aful07	7.974	**0.4628**	**0.7620**	9.676	0.5541	0.8087
Aful08	10.999	**0.5862**	**0.7801**	7.998	**0.5395**	**0.8181**
Aful10	4.000	0.1257	0.1278	3.852	0.0921	0.0896
Aful14	5.793	0.5875	0.6036	6.704	**0.4342**	**0.5645**
Aful17	13.991	0.8755	0.8063	16.683	0.8684	0.8986
Aful19	8.913	**0.7421**	**0.7882**	8.704	0.6579	0.7404
Aful20	9.566	**0.3420**	**0.4556**	12.903	**0.5139**	**0.8206**
Aful25	5.999	0.4493	0.4991	5.880	0.5200	0.6457
Aful28	13.789	0.8782	0.8738	13.865	0.8400	0.8832
Aful29	9.603	**0.8251**	**0.8241**	11.757	0.7733	0.8573
Aful30	8.000	**0.4444**	**0.6725**	7.983	**0.5132**	**0.8274**
Aful31	7.783	0.7330	0.8168	8.850	0.7632	0.8060
Aful33	5.843	**0.3808**	**0.5186**	6.000	0.5200	0.6984
Aful34	13.840	**0.8502**	**0.8989**	17.826	0.8333	0.8827
Aful35	9.770	0.7027	0.7238	8.977	0.8289	0.7531
Aful37	8.828	0.6930	0.7336	8.759	0.5200	0.5731
Aful38	5.999	**0.5597**	**0.5742**	6.868	0.5789	0.6730
Aful39	7.000	**0.3112**	**0.5970**	7.000	**0.5405**	**0.7128**
Aful41	10.707	**0.5681**	**0.6480**	8.868	0.6842	0.7413
Aful43	8.987	**0.7671**	**0.8034**	7.996	0.7368	0.8163
Aful44	18.568	**0.3793**	**0.8035**	13.749	**0.6111**	**0.8555**
Aful46	5.655	**0.3235**	**0.4744**	11.880	**0.5000**	**0.8199**
Aful49	8.000	**0.3140**	**0.6717**	8.859	**0.4865**	**0.7145**

(Table 2.2 continued)

	Anas fulvigula (*n* = 307)			*Anas platyrhynchos* (*n* = 76)		
Locus	AR	H_O	H_E	AR	H_O	H_E
Aful51	16.781	0.9173	0.8597	19.541	0.8919	0.9117
Aful55	9.000	**0.5724**	**0.7411**	8.970	**0.6269**	**0.7799**
Aful56	11.000	**0.7474**	**0.8534**	11.000	0.6818	0.8568
Aful57	3.608	0.1453	0.1454	6.640	0.2267	0.2120
Aful58	11.774	**0.5775**	**0.6582**	9.878	0.6267	0.6414
Aful61	39.967	**0.8274**	**0.9405**	34.821	**0.8158**	**0.9639**
Aful62	17.572	0.9086	0.9213	17.800	0.9079	0.9096
Aful64	7.850	**0.5055**	**0.6877**	6.759	**0.4133**	**0.6945**
Aful69	4.978	0.1014	0.1141	5.937	0.2000	0.2601
Aful81	4.832	**0.3908**	**0.5458**	5.784	**0.3108**	**0.5380**
Aful87	7.789	**0.3661**	**0.5220**	7.784	**0.4324**	**0.6382**
Mean (± std. error)	10.093 (± 1.039)	0.5609 (± 0.0383)	0.6620 (± 0.0348)	10.346 (± 0.937)	0.5845 (± 0.0341)	0.7086 (± 0.0350)

Mottled Ducks and Mallards had an excess of homozygotes at 22 and 18 loci, respectively, which contributed to deviations from Hardy-Weinberg equilibrium in both species. After adjusting for multiple comparisons via the Bonferroni method (Rice 1989), Mottled Ducks were still out of Hardy-Weinberg equilibrium at 22 loci, whereas Mallards were out of Hardy-Weinberg equilibrium at 13 loci (Table 2.2). Single locus deviations from Hardy-Weinberg expectations may have been the result of low sample size at some locations (e.g. Atchafalaya Delta WMA {n = 8}, Caernarvon {n = 2}, Mobile-Tensaw Delta {n = 5}, Pass-A-Loutre WMA {n = 8}), null alleles (Seyoum et al. 2012), and/or localized inbreeding. Localized inbreeding may occur as adult Mottled Ducks tend to be philopatric, and banded individuals are usually recovered in the same county as banded (Stutzenbaker 1988). Additionally, global (all loci combined) deviations from Hardy-Weinberg equilibrium by population could be the result of single loci or a small number of loci with extremely significant departures from Hardy-Weinberg expectations ($P < 0.001$) in certain sampling locations (Appendix 3). The F_{ST} and R_{ST} estimates between Mottled Ducks and Mallards were low (0.047 and 0.080, respectively) but statistically significantly. The PCA plot for Mottled Ducks and Mallards shows lower than expected structure, as axis one and two only explained 3.35% of the variation; however, individuals clearly form two distinct clusters and show obvious separation by species (Figure 2.2).

2.3.2 Genetic Mixture

STRUCTURE HARVESTER indicated that the genotypic data best fit a two-population model (Table 2.3). The distribution of q-values shows two distinct populations where a q-value near 0 indicates an individual to be a Mallard and a q-value near 1 indicates an individual to be a Mottled Duck (Figures 2.3, 2.4, and 2.5). A substantial difference in values for mean Ln $P(K)$ for

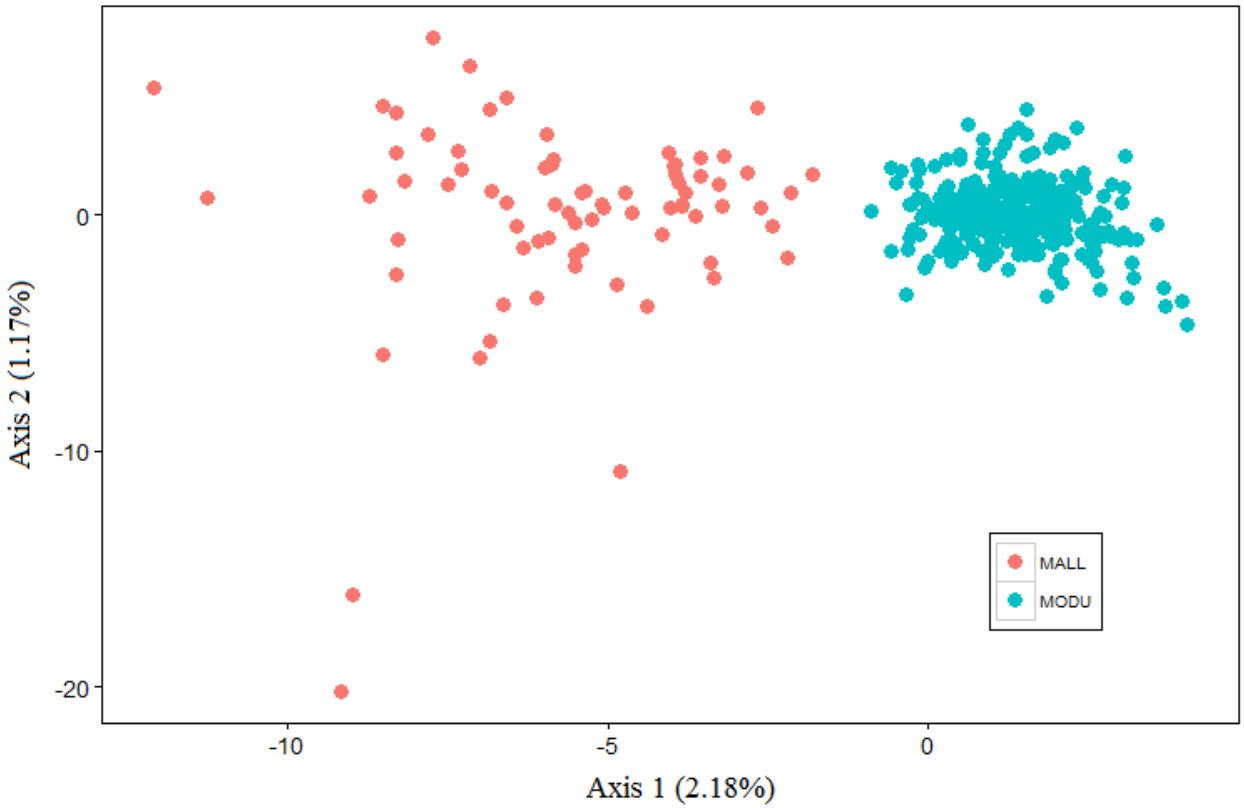

Figure 2.2 Principal Component Analysis plot produced in program R using the heirfstat package showing clustering of Mallards (MALL) and Mottled Ducks (MODU).

Table 2.3 Log probability of data as a function of successive *K* values ranging from 1 – 4 for Mottled Ducks, Mallards, and hybrids at 36 microsatellite loci using the admixture model implemented in the program STRUCTURE.

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-51066.5	0.3653	NA	NA	NA
2	10	-49744.8	1.5393	1321.71	483.5	314.1112
3	10	-48906.6	3.6752	838.21	382.91	104.187
4	10	-48451.3	3.2511	455.3	NA	NA

K = 1 and *K* = 2 populations (Table 2.3) indicates that there is model improvement from K = 1 to *K* = 2, suggesting that the best model is not *K* = 1.

Overall, 5.4% (22 out of 405) of ducks sampled in this study were deemed hybrids (*q* = 0.608 ± 0.056; Table 2.4). Ninety-five percent (303 out of 319) of putative Mottled Ducks were

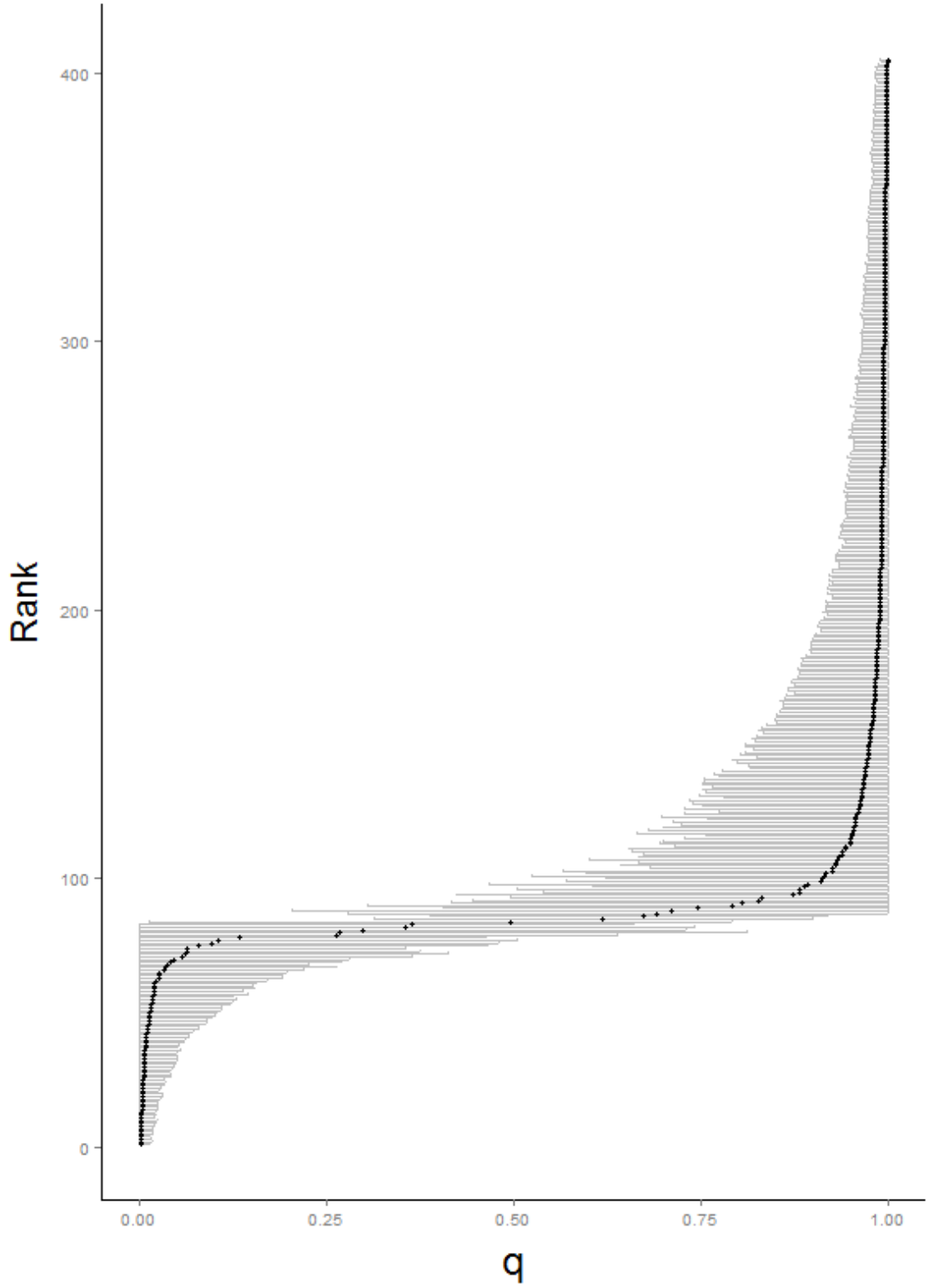

Figure 2.3 The distribution of the admixture proportion, *q*, among individuals. Individuals are ranked from the smallest to largest *q* (Rank), and are plotted against their respective *q* value. The horizontal bars indicate the 90% posterior probability surrounding each individual's *q*-probability. *Q*-values near zero are Mallards; values near one are Mottled Ducks.

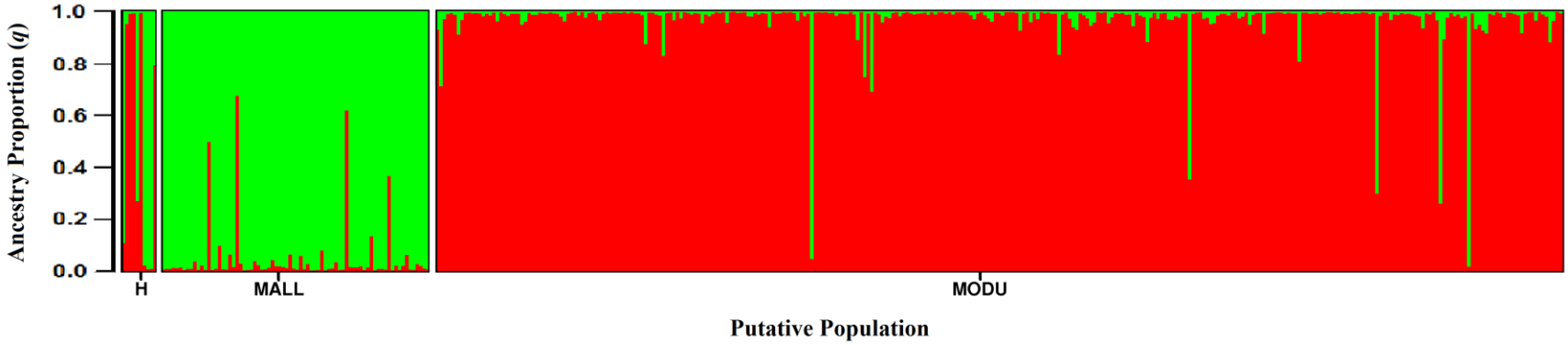

Figure 2.4 Population assignment bar graph generated in CLUMPAK based on 36 microsatellite loci for putative hybrids (H), Mallards (MALL), and Mottled Ducks (MODU).

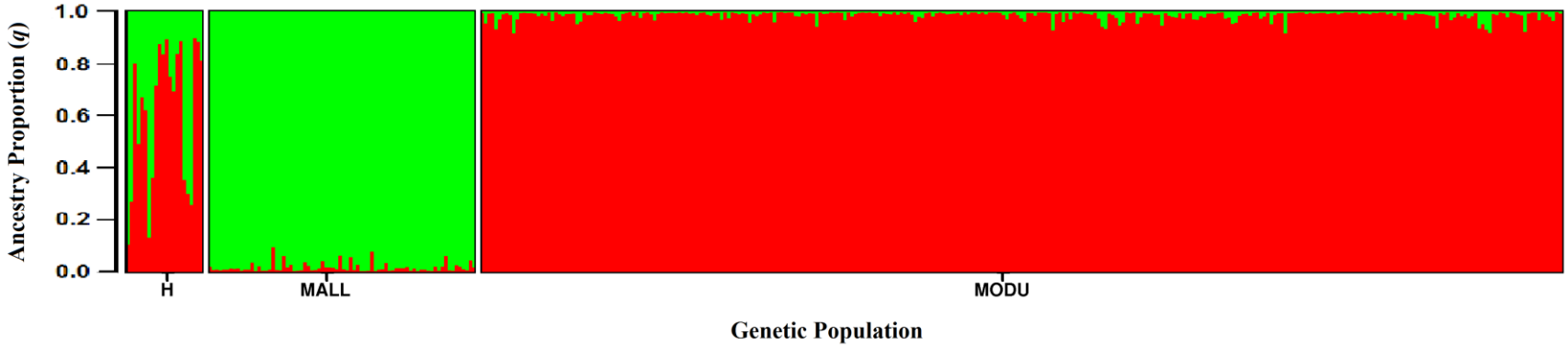

Figure 1.5 Population assignment bar graph generated in CLUMPAK based on 36 microsatellite loci for individuals genetically assigned as hybrids (H), Mallards (MALL), and Mottled Ducks (MODU).

Table 2.4 Genetic mixture assignment for hybrids, Mallards, and Mottled Ducks against their respective putative assignment via 36 microsatellite loci in the program STRUCTURE.

		Genetic Mixture Assignment		
Putative Species	*n*	hybrid	Mallard	Mottled Duck
hybrid	10	3 (30%)	3 (30%)	4 (40%)
Mallard	76	5 (6.6%)	71 (93.4%)	-
Mottled Duck	319	14 (4.4%)	2 (0.6%)	303 (95%)

assigned to one genetic cluster (q = 0.984 ± 0.001), whereas 93.4% (71 out of 76) of putative Mallards were assigned to the other cluster (q = 0.016 ± 0.002). Two putative Mottled Ducks were assigned as Mallards, but no putative Mallards were assigned as Mottled Ducks. Five Mallards were deemed hybrids; however, 42% and 48% of loci did not amplify for two of these individuals, which could produce an unreliable species assignment. Interestingly, only 30.0% of ducks thought to be hybrids upon collection (n = 10; Table 2.4) were genetically assigned as such. Four putative hybrids were assigned as Mottled Ducks and three were assigned as Mallards. Genotypic hybrids were collected from 12 out of 21 sampling locations with no obvious geographic distribution (Table 2.5). By state, hybrids occurred at 6.4% in Louisiana, 4.5% in Texas, 6.0% in Mississippi, with none in Alabama.

2.3.3 Hybrid Parentage

Mitochondrial DNA (mtDNA) sequences were used to assess hybrid maternal parentage. A total sequence length of 419 base-pairs was obtained for *ND2*, which resulted in seven haplotypes for Mottled Ducks, Mallards, and hybrids (Table 2.6). Out of seven haplotypes, two were unique for Mallards (haplotypes 6 and 7) and two were unique to hybrids (haplotypes 3 and 5). Haplotype 2 was the only haplotype that was shared between Mottled Ducks and hybrids, but

not Mallards. Haplotype 4 was shared between Mallards and hybrids, but not Mottled Ducks. Most *Cyt b* sequences were of poor quality, and were not used in the analysis.

Table 2.5 Number of hybrids detected from 405 ducks according to sampling location from Louisiana, Texas, Mississippi, and Alabama.

Sampling Location	No. Sampled	No. of Hybrids (%)
Louisiana		
Atchafalaya Delta WMA	10	2 (20.0)
Cameron-Prairie NWR	11	1 (10.0)
Marsh Island SWR	30	1 (3.3)
Ouachita WMA	7	1 (14.3)
Pass-A-Loutre WMA	11	3 (27.3)
Rockefeller SWR	36	2 (5.4)
Waverly	4	1 (25.0)
Other	62	0
Total	171	11 (6.4)
Texas		
Guadalupe Delta WMA	1	1 (100.0)
J.D. Murphree WMA	33	1 (3.0)
Justin Hurst WMA	79	3 (3.8)
Mad Island WMA	64	3 (4.5)
Other	0	0
Total	177	8 (4.5)
Mississippi		
Humphreys County	25	3 (12.0)
Other	25	0
Total	50	3 (6.0)
Alabama		
Mobile-Tensaw Delta	7	0
Other	0	0
Total	7	0
TOTAL	405	22 (5.4)

Table 2.6 Comparison of *ND2* gene haplotypes (419 bp) between Mottled Ducks (*n* = 14), Mallards (*n* = 10), and hybrids (*n* = 22). Abbreviations are as follows: single nucleotide polymorphism (SNP), base-pair (bp), Mottled Duck (MODU), and Mallard (MALL).

Haplotype	SNP (bp position)	No. of haplotypes found in MODU (%)	No. of haplotypes found in MALL *(%)*	No. of haplotypes found in hybrids (%)
1	-	7 (50%)	5 (50%)	10 (45.5%)
2	T (353)	7 (50%)	-	7 (31.8%)
3	C (80); G (405)	-	-	2 (9.1%)
4	T (128); C (179)	-	3 (30%)	2 (9.1%)
5	T (128); T (353)	-	-	1 (4.5%)
6	A (287)	-	1 (10%)	-
7	G (401)	-	1 (10%)	-

2.4 DISCUSSION

Western Gulf Coast Mottled Ducks do not appear to be in danger of losing their genetic identity through introgressive hybridization with Mallards. Only 4.4% of putative Mottled Ducks and 6.6% of putative Mallards were deemed hybrids (Table 2.4). Two putative Mallards were deemed hybrids but nearly half of loci did not amplify for these individuals. I observed during preliminary STRUCTURE analyses that using a small number of loci (*n* = 15) produced mixed-ancestry for all individuals, therefore these two individuals may have been misassigned as hybrids simply because an insufficient number of loci were genotyped. The remaining three specimens (two were male, one was female) were hunter harvested Mallards from Humphreys County, MS, and had nearly complete genotypes (at least > 94% of all loci amplified), which suggests that they were misidentified upon collection.

Two putative Mottled Ducks were assigned as Mallards in this study. One specimen, a female, was sampled during LDWF banding operations in July 2013 on Big Burns Marsh, LA

via brachial vein puncture. This specimen was released following banding and thus could not be analyzed for morphological hybrid characteristics (Chapter 3). The other specimen, a male from Justin Hurst WMA, TX, was collected in August 2012. This specimen (whole carcass) was available for hybrid key analysis (Chapter 3) and was correctly assigned as a Mallard or hybrid with the key, suggesting that the bird was misidentified in the field. Only three out of ten birds putatively identified as hybrids in the field were assigned as hybrids following genetic mixture analysis. Three were genetically assigned as Mallards, and four were assigned as Mottled Ducks, suggesting that there is confusion about the morphological and phenotypic characteristics that indicate a hybrid.

Anthropogenic changes to the natural landscape of the United States have been followed by a significant range expansion of North American Mallards (Brodsky and Weatherhead 1984; Mank et al. 2004; Kulikova et al. 2005). Consequently, previously allopatric species within the Mallard complex in North America (primarily the American Black Duck and Mexican Duck) now regularly interact and hybridize with Mallards. Furthermore, released game-farm Mallards that are not harvested or escape may account for the majority of non-migratory individuals that might be more inclined to hybridize with closely related species. In Florida, rates of hybridization between Mottled Ducks and Mallards are higher (~9.3%; Williams et al. 2005a) than those in the western Gulf Coast (~5.4%, this study).

Hybridization between Mottled Ducks and Mallards in the western Gulf Coast may be minimal due to the infrequency of interactions between the two species during the breeding season. Mottled Ducks are year-round residents of the western Gulf Coast, with a range that extends along the coast from Alabama to northeastern Mexico. Mottled Ducks in Louisiana and Texas are the most abundant breeding waterfowl species in the coastal marshes (Stutzenbaker

1988), nesting in coastal marsh and river delta habitats, but also in agricultural fields with lightly or ungrazed vegetation (Durham and Afton 2003). In contrast, Mallards are a migratory species that breeds throughout the United States (with a core breeding area concentrated in the prairie pothole region), except for sections of southeastern states and coastal Louisiana and Texas (Baldassarre 2014). Furthermore, Mallards are rarely observed in coastal habitats during the western Gulf Coast Mottled Duck survey conducted annually in early April (L. Reynolds, LDWF, personal communication), thus, potential encounters and probability of hybridization between the two species may be low.

In Florida, hybridization appears to be a more serious threat due to increasing occurrences of non-migratory Mallards and the habits of Florida Mottled Ducks. Mottled Ducks in Florida inhabit the peninsula south of Alachua County (Bielefeld et al. 2010), but are most common in the wetlands of Lake Okeechobee and the Everglades Agricultural Area (Baldassarre 2014). However, unlike Gulf Coast Mottled Ducks, Florida Mottled Ducks will inhabit ditches, ponds on ranches and farms (Johnson et al. 1991), and irrigation reservoirs associated with citrus crops (Bielefeld and Cox 2006). It is estimated that over half of Florida Mottled Ducks may use urban areas where high concentrations of Mallards also congregate (Bielefeld et al. 2010). Additionally, Mottled Ducks captured in urban/suburban areas showed a propensity towards occupying artificial ponds and ditches (Bielefeld 2011), which may elevate hybridization risks because Florida Mottled Ducks are more likely to encounter and hybridize with non-migratory Mallards in urban areas (Florida Fish and Wildlife Conservation Commission 2011).

It is unclear whether hybridization between Mottled Ducks and Mallards in the western Gulf Coast will be a conservation issue in the future. In both the western Gulf Coast and Florida, habitat may be the driving force for Mottled Duck survival. Indeed, participants of the Gulf

Coast Joint Venture Mottled Duck Working Group meeting (August 2003) overwhelmingly favored spring and summer habitat loss as the leading factor limiting the survival and recruitment of western Gulf Coast Mottled Ducks (Wilson 2007) because estimates of coastal marsh loss are large: 487,695 hectares were lost in Louisiana from 1932 – 2010 (Couvillion et al. 2011), and 320,000 hectares were lost in Texas since the 1950s (Moulton et al. 1997).

However, habitat loss and/or the unavailability of wetlands may also indirectly cause hybridization. As Mottled Duck coastal habitat is compromised or lost, Mottled Ducks may move to remaining stable wetland habitats, such as those found in urban areas where non-migratory Mallards are more likely to congregate. For example, in Florida, Mottled Ducks in the Upper St. Johns River Basin (USJRB) moved from rural wetlands to wetlands associated with urban areas in response to reduced wetland availability (Bielefeld and Cox 2006). Moreover, Bielefeld and Cox (2006) found that the majority of Mottled Ducks that moved into urban areas remained there following improved conditions in the USJRB the following year. Once Mottled Ducks move to urban areas, they may remain there, and only occasionally move between urban and coastal habitat, and thus limit hybridization to urban habitats. For example, Varner et al. (2014) found that female Mottled Ducks in Florida seldom (6%) move between urban and rural areas (coastal habitat is not occupied), suggesting that hybridization may be restricted to ducks that occupy urban areas. In the western Gulf Coast, Mottled Ducks seem to principally inhabit coastal marsh and avoid urban areas. However, habitat loss and the unavailability of coastal wetlands in the future may force Mottled Ducks to venture into urban habitats and potentially hybridize with non-migratory Mallards.

Although extensive hybridization between two distinct species is undesirable, historical hybridization between North American Mallards and western Gulf Coast Mottled Ducks may

have increased genetic diversity in western Gulf Coast Mottled Ducks. Peters et al. (2014) found nearly as much genetic diversity in western Gulf Coast Mottled Ducks at nuclear DNA as North American Mallards, although Mallards have a much higher population census size than Mottled Ducks (11.6 million Mallards; Zimpfer et al. 2015, versus ~159,000 Mottled Ducks; USFWS 2015), and a higher effective population size (N_e = 2,400,000 Mallards and 120,000 Mottled Ducks; Peters et al. 2014). High genetic diversity in western Gulf Coast Mottled Ducks may have occurred due to some gene flow over the long-term between the two species (Peters et al. 2014).

Mallards, American Black Ducks, Mexican Ducks, and Mottled Ducks represent the 'Mallard complex' within North America; therefore, a comparison of hybridization between these species could provide additional context to hybridization rates between Mottled Ducks and Mallards. Mallards expanded their range eastwardly following habitat alteration and then colonized the eastern territories previously dominated by American Black Ducks, particularly during the 1960s and 1970s (Mank et al. 2004). Mank et al. (2004) found that several decades of introgressive hybridization between American Black Ducks and newly-arrived Mallards have eroded genetic differentiation between the two species. G_{ST} estimates, a measure of genetic divergence between populations, have decreased substantially as G_{ST} values for Black Duck-Mallard museum samples collected prior to 1940 was 0.146, but only 0.008 for modern samples collected in 1998 (Mank et al. 2004). Another closely related species to Mottled Ducks are Mexican Ducks. Mexican Ducks reside in the southwestern portion of the United States and are possibly the least studied waterfowl species in North America (Williams 1980). The genetic structure of Mexican Ducks or genetic mixture analysis involving Mallards has not been studied extensively; however, McCracken et al. (2001) found that Mexican Ducks are genetically similar

to Mallards (using only four samples), likely due to historical hybridization. Lavretsky et al. (2014) could not discern phylogenetic relationships between Black Ducks, Mexican Ducks, and Mallards using nuclear DNA; however, Mexican Ducks and Mallards could be differentiated with mtDNA. One explanation is that male Mallards hybridize with female Mexican Ducks, and hybrids assimilate and backcross into Mexican Duck populations (Lavretsky et al. 2014).

Asymmetric introgression is a phenomenon observed between Mallards and Mottled Ducks in Florida, where there is more gene transfer from Mallards into the Mottled Duck genome than vice versa (Williams et al. 2005a). Kulikova et al. (2004) found similar results in eastern Russia where there was more Mallard mtDNA gene transfer into the Eastern Spot-billed Duck (*Anas zonorhyncha*) genome. Despite apparent asymmetric introgression between Mallards and other species globally, there is no evidence for such a phenomenon in the western species Gulf Coast as similar levels of putative Mottled Ducks and Mallards were hybrids (Table 2.4).

One mitochondrial gene (*ND2*) amplified reliably and could be used to examine patterns of maternal parentage in hybrids, as some haplotypes were unique to either Mottled Ducks or Mallards, a result observed in this study as well as others. In this study, haplotype 2 was present in Mottled Ducks but not Mallards, and haplotypes 4, 6, and 7 were present in Mallards but not Mottled Ducks (Table 2.6). An NCBI blast search (Altschul et al. 1997) for haplotype 2 had a 100% match with a Mottled Duck sequence (GenBank accession #AF059134.1) but did not exactly match any Mallards sequences. Similarly, a blast search for haplotype 4 had a 100% match with multiple Mallard sequences (e.g. GenBank accession #KJ883269.1), but did not match any Mottled Ducks sequences. Thus, the seven hybrids with haplotype 2 could be the offspring of male Mallard x female Mottled Duck interbreeding and the two hybrids with haplotype 4 could be the offspring of male Mottled Duck x female Mallard interbreeding. No

other haplotype relationships can be inferred because they were either shared by both Mottled Ducks and Mallards (haplotype 1), were only found in Mallards and hybrids (haplotypes 6 and 7), or were only found in hybrids (haplotypes 3 and 5).

Ultimately, genetic mixture analysis of western Gulf Coast Mottled Ducks and North American Mallards provided an important insight for the conservation of western Gulf Coast Mottled Ducks. First, despite introgressive hybridization in Florida populations, rates of hybridization in western Gulf Coast Mottled Ducks appear to be low and should be of limited conservation concern. Despite apparent asymmetric introgression between Mallards and Florida Mottled Ducks, a similar percentage of putative Mallards and Mottled Ducks were hybrids in this study. Monitoring of hybridization between these two species in the Gulf Coast will be critical to ensure rates of hybridization do not increase as a consequence of habitat modification.

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CHAPTER 3. VALIDATION OF A PLUMAGE KEY DISTINGUISHING FLORIDA MOTTLED DUCKS FROM MALLARDS AND THEIR HYBRIDS FOR WESTERN GULF COAST MOTTLED DUCKS

Mottled Ducks (*Anas fulvigula*) and Mallards (*A. platyrhynchos*) can be difficult to distinguish because the females of both species resemble each other throughout the year Additionally, male Mallards in basic plumage that are transitioning into pre-alternate plumage could have feather characteristics indicative of hybrids (e.g. green feathers on head) and may be confused with Mottled Ducks or hybrids. Finally, it may be more difficult to distinguish Mottled Ducks from hybrids because hybrid offspring of experimentally controlled Mottled Duck x Mallard crosses apparently resemble Mottled Ducks (Stutzenbaker 1988). Although Mottled Ducks, Mallards, and hybrids can be distinguished genetically (Chapter 2), a genetic analysis is not practical in the field if biologists and managers need to cull hybrids or Mallards to prevent hybridization.

Recently, a key, entitled, "A Key to Distinguish Florida Mottled Ducks from Mallards and their Hybrids," (hereafter "MODU key") was created by Bielefeld et al. (in review). Museum specimens were used to develop the MODU key in order to identify useful traits for separating Florida Mottled Ducks from Mallards and their hybrids. Additionally, contemporary specimens of known genetic identity were used for verification of the MODU key. Overall, the MODU key has proven to be greater than 90% effective for distinguishing Florida Mottled Ducks from Mallards and their hybrids.

In this chapter, I compared genetic and key assignments to examine the efficacy of the MODU key for western Gulf Coast Mottled Ducks.

3.1 METHODS

I used the MODU key to identify 135 duck whole carcasses or wings using feather characteristics on the head, wings, body, and tail, without *a priori* genetic information (Table 3.1). The MODU key was provided in a laminated booklet with pictures showing representative feather characteristics for Mottled Ducks and hybrids/Mallards.

The MODU key consists of five sub-keys for males and four for females, each including wing or no-wing assessments. Overall, 12 plumage traits were evaluated; for males, each sub-key consisted of two to five plumage traits, whereas one to seven plumage traits were evaluated for females in each sub-key. In males, the first four sub-keys included feather characteristics that automatically assigned a specimen as a hybrid or Mallard. These sub-key feather characteristics consisted of any green feathers on the head, more than 3 mm of white on the greater coverts (GCWoW), central tail feathers showing any degree of curl, and under-tail coverts with black or dark brown spots. The final sub-key for males used a body feather index (BFS; excluded wings) which scored each Mottled Duck or hybrid/Mallard feather characteristic, where a BFS of one or less was indicative of a Mottled Duck and a score equal to two or more was indicative of a hybrid or Mallard. For this sub-key, feather characteristics indicative of hybrids/Mallards were evaluated including: any green feathers on head, gray and/or vermiculated flank feathers (Mottled Ducks having a buff coloration with chevron pattern), spotted or black under-tail covert pattern (Mottled Ducks having a buff coloration with chevron pattern), and slightly or fully curled central tail feathers (Mottled Ducks having flat central tail feathers).

In females, the first three sub-keys included feather characteristics that automatically assigned a specimen as hybrid or Mallard. These feather characteristics consisted of > 4 mm of white on the 5th secondary trailing the speculum (WoTEW), ≥ 4 of white on the WoTEW, and

Table 3.1 Number and type of sample used for morphological identification of ducks using an experimental key to distinguish Mottled Ducks from Mallards and their hybrids.

Location	*n*	Type of Sample
Big Burns Marsh, LA	4	Whole Carcass
Caernarvon, LA	1	Whole Carcass
Cameron-Prairie NWR, LA	3	Whole Carcass
Guadalupe Delta WMA, TX	1	Whole Carcass
J.D. Murphree WMA, TX	33	Whole Carcass
Justin Hurst WMA, TX	23	Whole Carcass
Mad Island WMA, TX	43	Whole Carcass
	22	Wing Only
Mobile-Tensaw Delta, AL	5	Wing Only

light colored under-tail coverts with dark brown spots. The final sub-key for females was a phenotype score (PS) which scored each Mottled Duck or hybrid/Mallard feather characteristic, where a score of 1 or less was indicative of a Mottled Duck and a score equal to 2 or more was indicative of a hybrid or Mallard. For this sub-key, additional feather characteristics indicative of hybrids/Mallards were evaluated including: greater coverts showing any white, a distinct blue or purple speculum (Mottled Ducks have a green speculum), solid or grey lesser coverts (Mottled Ducks have a buff edge), solid color on the leading edge of the first primary feather (Mottled Ducks show mottling), and a white or very light outer edge on the outer two tail feathers (Mottled Ducks have dark buff edge). For both species, if only a single hybrid/Mallard feather characteristic was present, it was considered a hybrid (regardless of the BFS or PS key score). For example, some male specimens may have had a GCWoW greater than 3 mm, but their BSI was zero – these specimens were considered hybrids/Mallards.

The MODU key evaluates each sex class separately, thus, the sex of putative Mottled Ducks, Mallards, and hybrids was determined by PCR and gel electrophoresis using primers that simultaneously amplify the CHD1 gene from both sex chromosomes (detailed in Peters et al. 2014). On agarose gels, females were identified by the presence of two bands (representing the Z and W chromosomes) whereas males were identified by one band (representing the Z chromosome). PCR amplification consisted of 1 minute at 94°C for initial denaturation, followed by 45 cycles of 20 seconds at 94°C, 20 seconds at 58°C, and 20 seconds at 72°C for elongation, ending with a final elongation step of 72°C for 7 minutes. After amplification, 2 µl of PCR product was mixed with 2 µl v/v EZ-Vision Three (Ameresco, Solon, Ohio), electrophoresed in 1.2% agarose at 150 volts for 30 – 35 minutes, and viewed with Kodak Molecular Imaging software (Version 5.0, Rochester, New York) for bands.

Each specimen was frozen at -20°C until examined for hybrid characteristics, without prior information regarding genetic identity. Each specimen was thawed and dried in order to clearly identify feather characteristics (e.g. green feathers on head, central tail feather curl). After assessing plumage characteristics, each specimen's MODU key assignment was compared to its genetic assignment.

3.2 RESULTS

In total, 135 ducks were sexed, keyed, and genotyped. Overall, 29.63% (40 out of 135) of the ducks were female and 70.37% (95 out of 135) were male. The MODU key correctly assigned 97% of ducks (131 out of 135) to each species, and only 3% of ducks were misassigned. By sex, 95% (38 individuals) of females and 97.9% (93 individuals) of males were correctly assigned.

3.3 DISCUSSION

The MODU key assignments were consistent with genetic assignments for 131 out of 135 specimens. The accuracy of the MODU key for distinguishing Mottled Ducks from Mallards and their hybrids in the western Gulf Coast is similar to its success in Florida where it was developed. Implementing several sub-keys for both sexes greatly increases the ability to identify a hybrid in the field, regardless of the condition of the specimen. Indeed, Bielefeld et al. (in review) highlight the flexibility of the MODU key during management operations, such as banding, when Mottled Ducks are in pre-basic molt and wing feathers are not always available for assessment.

Genetic species assignments were contradictory to MODU key assignments in only four specimens. Of these four, three were keyed as Mottled Ducks but were genotyped as hybrids (Table 3.2). One specimen, a female from Mad Island WMA, TX (#78), only had a wing available for evaluation, thus, only wing characteristics could be analyzed and no other important potential hybrid characteristics on other parts of the body could be evaluated (Figure 3.1a). For example, if this specimen had a spotted under-tail covert pattern in combination with another hybrid characteristic, such as a white or very light outer edge on the outer two tail feathers, then this would have classified the specimen correctly as a hybrid based on its PS score. Two specimens, a female from J.D. Murphree WMA, TX (#35; Figure 3.1b) and a male specimen from Justin Hurst WMA, TX (#305), were misassigned as Mottled Ducks because they did not show any hybrid characteristics. Of the five specimens that were misassigned, one was keyed as a hybrid or Mallard but was genotyped as a Mottled Duck (#165). This specimen, a male from Justin Hurst WMA, TX, showed no hybrid characteristics, except the width-of-white on the greater coverts which measured 4 mm (greatly exceeding the threshold of 3 mm), which alone

Table 3.2 Specimens for which morphological identification did not match genetic assignment tests. Abbreviations are as follows: Mottled Duck (MODU), Mallard (MALL), Hybrid (H), Female (F), Male (M), and Greater Covert Width-of-Whilte (GCWoW).

Sample I.D.	Putative Assignment	Sample Type	Sex	Location	MODU Key Assignment	Genetic Mixture Assignment	Reason for incorrect assignment
35	MODU	Whole Carcass	F	J.D. Murhpree WMA, TX	MODU	H	No hybrid characteristics
78	MODU	Wing	F	Mad Island WMA, TX	MODU	H	Wing only; no hybrid characteristics
165	H	Whole Carcass	M	Justin Hurst WMA, TX	H or MALL	MODU	GCWoW = 4 mm; no other hybrid characteristics
305	MODU	Whole Carcass	M	Justin Hurst WMA, TX	MODU	H	No hybrid characteristics; carcass extremely bloody

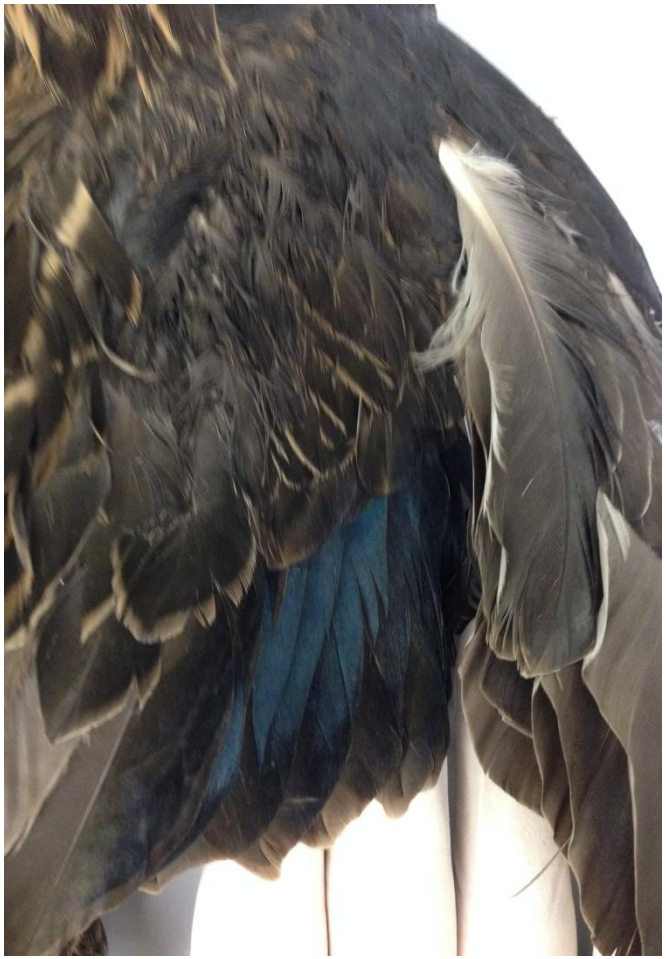
a)  **b)** 

Figure 3.1 a) Misassigned specimen #78, a female from Mad Island WMA, TX - wing sample; keyed as a Mottled Duck but genetically assigned as a hybrid or Mallard. Note the white on the trailing edge of the speculum is minimal (< 4 mm), and buff edges to lesser coverts, mottling on the leading edge of the 1st primary feather, and green speculum coloration which all indicates a Mottled Duck. b) Misassigned specimen #35, a female from J.D. Murphree WMA, TX – whole carcass sample; keyed as a Mottled Duck but genetically assigned as a hybrid or Mallard.

classified it as a hybrid or Mallard (Figure 3.2). It is possible that this specimen was a complex backcross between Mottled Ducks and Mallards with > 90% Mottled Duck genetic ancestry. All specimens genotyped as Mallard that were available for morphological key evaluation (6 individuals) were correctly assigned as Mallard or hybrids.

Two putative Mottled Ducks were incorrectly identified in the field, as key and genetic assignments indicated that both were Mallards. One specimen, a female, was sampled during LDWF banding operations in July 2013 on Big Burns Marsh, LA via brachial vein puncture.

Figure 3.2 Misassigned specimen #165; keyed as a hybrid or Mallard but genetically assigned as a Mottled Duck. Note the greater covert width-of-white above the speculum, measuring greater than 3 mm, classifying it as a hybrid or Mallard.

This specimen was released following banding and thus could not be analyzed for morphological characteristics. The other specimen, a male from Justin Hurst WMA, TX, was collected in August 2012. This specimen (whole carcass) was available for morphological key evaluation and was correctly keyed as a Mallard or hybrid based on spotted under-tail coverts and a greater covert width-of-white of 11 mm.

Ten ducks were labeled as hybrids in the field by the collector; however, only three of these birds were assigned as hybrids following genetic mixture analysis. This would suggest that there is variable plumage in Mottled Ducks and hybrids that may lead to misidentifications. This seems especially true for the variation in width-of-white on the greater coverts and white trailing the speculum, which are the characteristics that produce the most disagreement among mangers (B. Davis, Wisconsin DNR; personal communication). Some managers may declare that any white bordering the speculum is indicative of a hybrid, whereas others may allow for some variation in the width-of-white. All 10 putative hybrids were available for key analysis (Table 3.3). Of the three putative hybrids that were genotyped as hybrid, two were correctly keyed as hybrid. As previously discussed in this section, one putative hybrid that was genotyped as Mottled Duck was keyed incorrectly as a hybrid based on the width-of-white on the greater coverts measuring 4 mm (Figure 3.2). All putative hybrids that were genotyped as Mallards were correctly keyed as hybrid or Mallard. Feather characteristics that distinguish Mottled Ducks from Mallards and their hybrids (Bielefeld et al., in review) are valuable to waterfowl managers not only in peninsular Florida but in the western Gulf Coast, because Mallards and hybrids can now be reliably identified during summer management operations if culling is desired.

Overall, the MODU key developed for distinguishing Florida Mottled Ducks from Mallards and their hybrids has proven to be effective (97%) for ducks in the western Gulf Coast.

Table 3.3 Information for ten specimens identified as hybrids in the field (putative), including sample type, sex, location, assignment based on the MODU key, assignment based on genetic mixture analysis, and reason for MODU key assignment (if applicable). Abbreviations are as follows: Mottled Duck (MODU), Hybrid (H), Mallard (MALL), Female (F), Male (M), and Greater Covert Width-of-White (GCWoW).

Sample I.D.	Putative Assignment	Sample Type	Sex	Location	MODU Key Assignment	Genetic Mixture Assignment	Reason for MODU key assignment
22	H	Whole Carcass	M	Mad Island WMA, TX	H	H	GCWoW = 9 mm; spotted under-tail coverts
23	H	Whole Carcass	M	Mad Island WMA, TX	MODU	MODU	No hybrid or MALL characteristics
25	H	Whole Carcass	M	Mad Island WMA, TX	MODU	MODU	No hybrid or MALL characteristics
26	H	Whole Carcass	M	Mad Island WMA, TX	MODU	MODU	No hybrid or MALL characteristics
163	H	Whole Carcass	M	Guadalupe Delta WMA, TX	MODU	H	No hybrid or MALL characteristics; undergiong molt
165	H	Whole Carcass	M	Justin Hurst WMA, TX	H or MALL	MODU	GCWoW = 4 mm
171	H	Whole Carcass	M	J.D. Murphree WMA, TX	H or MALL	MALL	Molting bird; GCWoW = 11 mm; spotted under-tail coverts
412	H	Whole Carcass	M	Big Burns Marsh, LA	H or MALL	MALL	Multiple hybrid or MALL feather characteristics (BSI = 5)
414	H	Whole Carcass	F	Big Burns Marsh, LA	H or MALL	MALL	Multiple hybrid or MALL feather characteristics (PS = 5)
416	H	Whole Carcass	M	Cameron-Prairie NWR, LA	H or MALL	H	GCWoW = 4 mm

If western Gulf Coast Mottled Duck managers want to remove hybrid ducks during management activities, such as banding operations, the MODU key will be useful for obtaining correct identifications.

3.4 LITERATURE CITED

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CHAPTER 4. POPULATION STRUCTURE OF WESTERN GULF COAST MOTTLED DUCKS

Two distinct subspecies of Mottled Ducks are recognized by the 1957 edition of the American Ornithologists' Union's *Checklist of North American Birds*, the most recent edition to include sub-species: one subspecies occurs in peninsular Florida (*Anas fulvigula fulvigula*) and the other, the focus of this study, is a resident of the Gulf Coast from Alabama to northeastern Mexico (*A. f. maculosa*; McCracken et al. 2001, Williams et al. 2005b; Wilson 2007). Evidence for two distinct subspecies includes band recovery data, which shows that no Mottled Ducks banded from 1950 – 2010 in Texas (n = 26,762) or Louisiana (n = 37,561) were recovered in Florida. Likewise, 99.4% of Mottled Ducks banded in Florida from 1950 – 2010 (19,294) were recovered in Florida, with none of the remaining 0.6% recovered in Texas or Louisiana (Baldassarre 2014).

Genetic data also support two distinct Mottled Duck subspecies. McCracken et al. (2001) used an analysis of molecular variance (AMOVA) and found no evidence of genetic mixture between Florida Mottled Ducks and western Gulf Coast Mottled Ducks from Louisiana and Texas based on mtDNA haplotypes, suggesting that gene flow is undetectable or does not occur between the two Mottled Duck subspecies. Similarly, Williams et al. (2005b) found significant genetic differentiation between Florida and Texas Mottled Duck populations using allozyme and microsatellite loci. Rare gene flow, coupled with the non-migratory nature of Mottled Ducks and the geographical distance between Florida and western Gulf Coast populations, seems to confirm the subspecies status of these two populations. Given that broad scale genetic structure exists, genetic structuring at finer-scales may also exist; however, no information is available to evaluate this for western Gulf Coast Mottled Duck population.

Mottled Ducks may show genetic structuring in the western Gulf Coast due to restricted bird movements or barriers to gene flow. For example, narrow home ranges of Mottled Ducks or low dispersal could limit gene flow across the western Gulf Coast. Stutzenbaker (1988) stated that adult Mottled Ducks make small movements along the Gulf Coast, and band recoveries are often in the same county as banded. Of 4,564 recoveries of Louisiana banded Mottled Ducks from 1950 – 2010, 90.9% were recovered in Louisiana, 8.9% were in Texas, and none in Mexico. In Texas, most (76.7%) of the 3,547 recoveries of banded Mottled Ducks were in Texas, however, 22.2% were recovered in Louisiana, and 0.9% were in Mexico (Baldassarre 2014). This seems to indicate that there is considerable dispersal of Mottled Ducks between Texas and Louisiana; however, most of the Texas bands recovered in Louisiana and most of the Louisiana bands recovered in Texas were from birds banded near the border, within the Chenier Plain Gulf Coast Joint Venture Initiative area (W. Selman, LDWF, unpublished data).

Additionally, restricted gene flow among western Gulf Coast Mottled Duck populations could arise due to barriers, such as lack of continuous suitable breeding habitat. This may occur where large cities (e.g. Houston, Texas) interrupt Mottled Duck habitat or where public development (e.g. housing, beaches) disrupts contiguous habitat (particularly along the Mississippi coast). Barriers to gene flow may increase genetic drift resulting in a loss of alleles. Identifying barriers to gene flow could help managers to detect areas where Mottled Duck populations may have reduced genetic variation. Accordingly, managers will be able to make more informed conservation decisions if they understand gene flow, and possible geographic barriers, between populations.

In this chapter, I assessed the number of genetically distinct Mottled Duck populations and estimated migration rates separately regions of Mottled Ducks in the western Gulf Coast.

4.1 METHODS

Sampling and microsatellite genotyping methods are identical to those described in Chapter 2. Population structure and genetic diversity analysis were based on 307 Mottled Ducks with > 90% Mottled Duck ancestry as identified in the STRUCTURE results from Chapter 2.

4.2 STATISTICAL ANALYSES

4.2.1 Genetic Diversity

Genetic diversity was measured as observed (H_O) and expected heterozygosities (H_E), and allelic richness (AR) by Mottled Duck sampling location in program R using the hierfstat package (Goudet 2005). Inbreeding coefficients (F_{IS}) were calculated using GENEPOP version 4.3 (Rousset 2008).

4.2.2 Population Structure

In STRUCTURE (Pritchard et al. 2000), models ranging from a single-population model to a 14-population model (K = 1 – 14) were tested using 10 replications for each model, a burn-in of 200,000 steps, followed by 1,000,000 MCMC iterations. The admixture ancestry model and correlated allele frequencies were assumed among populations. To determine the most likely number of clusters (K) in the overall sample, output from STRUCTURE was used in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012) which evaluates the likelihood of each model and selects the best K using the Evanno method (Evanno et al. 2005). Finally, output from STRUCTURE was used in the program CLUMPAK (Kopelman et al. 2015) to create bar graphs for individuals according to their ancestry proportions (q) by population. No prior information regarding sampling location was included in these analyses.

Genetic structure for western Gulf Coast Mottled Duck sampling locations was also examined by estimating a global F_{ST} using an analysis of molecular variance (AMOVA) in ARLEQUIN version 3.5 (Excoffier and Lischer 2010). In ARLEQUIN, statistical significance was calculated using 20,000 randomizations and a significance level of 0.05. Pairwise estimates of F_{ST} between Gulf Coast Mottled Duck sampling locations were performed in R using the hierfstat package (Goudet 2005). Significance of pairwise F_{ST} estimates was based on 95% confidence intervals using bootstrapping across loci, where estimates bracketing zero are not significant. Pairwise estimates of R_{ST} were calculated in GENEPOP version 4.3 (Rousset 2008). R_{ST} estimates take into account the identity (length) of alleles at microsatellite loci, whereas F_{ST} estimates do not and can under-estimate population differentiation; for instance, a low F_{ST} value was calculated between two races of shrew at a Y-chromosome microsatellite locus even though the two races did not share any alleles at that locus (Brunner and Hausser 1996; Balloux et al. 2000). Additionally, a two-dimensional Principal Component Analysis (PCA) between western Gulf Coast Mottled Duck sampling locations was implemented in R using the hierfstat package (Goudet 2005) by plotting each individual using their respective microsatellite allele composition without prior sampling location designation.

Gene flow was inferred by determining the number of migrants between regions of western Gulf Coast Mottled Ducks with the program MIGRATE-N version 3.6.11 (Beerli and Felsenstein 2001; Beerli 2006). First, western Gulf Coast Mottled Ducks were grouped into three regions including a western region (including Guadalupe Delta WMA, TX, Mad Island WMA, TX, and Justin Hurst WMA, TX), a central region (including J.D. Murphree WMA, TX, Cameron-Prairie NWR, LA, Big Burns Marsh, LA, Rockefeller SWR, LA, and Marsh Island SWR, LA), and an eastern region (including Atchafalaya Delta WMA, LA, Point aux Chenes

WMA, LA, Caernarvon, LA, Pass-a-Loutre WMA, LA, and Mobile-Tensaw Delta, AL). These regions corresponded to habitat types and Gulf Coast Joint Venture initiative areas including the Texas Mid-Coast (western region), the Chenier Plain (central region), and both the Mississippi River Coastal Wetlands and Coastal Mississippi-Alabama (eastern region).

The Texas Mid-Coast initiative area occurs from Corpus Christi to Galveston Bay, Texas, and is comprised of restricted estuarine systems associated with seagrass beds and subtidal aquatic bed wetlands, and is less extensive than the chenier and delta marshes in western and eastern Louisiana, respectively (Wilson and Esslinger 2002). The Chenier Plain initiative area extends from Galveston Bay, Texas to Vermillion Bay, Louisiana, and is composed of salt, brackish, intermediate, and fresh marsh habitats that are associated with beach ridges, known as cheniers, that parallel the coastline and form natural levees bordering immense marsh habitat (Esslinger and Wilson 2001). Marsh Island WMA was included in the central region even though it is a part of the Mississippi River Coastal Wetlands initiative because it is currently thought to have habitat similar to the Chenier Plain (W. Selman, LDWF, personal communication). The Mississippi River Coastal Wetlands and Coastal Mississippi-Alabama initiative areas extend from Vermillion Bay, Louisiana, to Perdido Bay on the Alabama-Florida border. The Mississippi River Coastal Wetlands initiative area includes widespread marsh habitat with numerous large, open water bays and two major fresh marsh river-deltas: the Mississippi and the Atchafalaya (Wilson et al. 2002). The Coastal Mississippi-Alabama initiative area includes brackish to saline marsh and coastal pine flatwoods in Mississippi, and mostly large bays, most notably Mobile Bay, and estuary systems with some forested wetlands in Alabama (Manlove et al. 2002).

A second grouping strategy split western Gulf Coast Mottled Ducks into two regions including a western region (including Guadalupe Delta WMA, TX, Mad Island WMA, TX, and

Justin Hurst WMA, TX), and an eastern region (including J.D. Murphree WMA, TX, Cameron-Prairie NWR, LA, Big Burns Marsh, LA, Rockefeller SWR, LA, Marsh Island SWR, LA, Atchafalaya Delta WMA, LA, Point aux Chenes WMA, LA, Caernarvon, LA, Pass-a-Loutre WMA, LA, and Mobile-Tensaw Delta, AL). These regions were selected to determine whether gene flow in the western Gulf Coast occurred across a habitat gradient: habitat quality is thought to be lower for Mottled Ducks in the western region (in the Texas Mid-Coast) and higher in the eastern region (Chenier Plain and delta habitats including the Atchafalaya and Mississippi; W. Selman, LDWF, personal communication). Thus, we would expect to find more individuals migrating into higher quality habitat in the eastern region and fewer individuals migrating into lower quality habitat in the western region.

MIGRATE-N can use either a maximum likelihood or Bayesian inference approach to estimate mutation-scaled effective population size ($\theta = 4N_e\mu$, where N_e is the effective population size and μ is the mutation rate) and mutation-scaled effective migration rates ($M = m/\mu$, where m = migration rate) with genetic data. I used microsatellite data to estimate the number of immigrants with Bayesian inference using a migration matrix model (which included estimation of all θ and M parameters, except between the western and eastern regions in the three region model), where the mutation rate was kept constant for all loci and missing data were excluded. I used a Brownian motion model with the following search parameters: θ and M were estimated using F_{ST}, MCMC runs used one long chain, recording every 100 steps, and an uniform prior distribution, specifying the minimum, maximum, and delta values for each parameter group: population size (Θ) and migration rate (M). For Θ, minimum, maximum, and delta values were set to 0, 100, and 10, respectively, as initial analyses indicated that a maximum of at least 100 for Θ was appropriate for microsatellite data. For M, minimum, maximum, and

delta values were set to 0, 10,000, and 1,000 to ensure that the upper prior boundary was not too low (as indicated by preliminary runs). Each model was replicated ten times and averaged results were calculated to obtain mean mutation-scaled effective population size and mutation-scaled effective migration rates. Estimates provided in the results section are means ± standard error.

4.3 RESULTS

4.3.1 Genetic Diversity

Mean observed and expected heterozygosities for Mottled Ducks by sampling locations were 0.5609 ± 0.0099 and 0.6578 ± 0.0054, respectively (Table 4.1). The mean allelic richness for locations with fewer than 10 individuals and those with 10 or greater individuals was 1.654 ± 0.005 and 4.759 ± 0.023, respectively. The mean inbreeding coefficient (F_{IS}) across 12 Mottled Duck sampling locations was 0.1461 ± 0.0119.

4.3.2 Population Structure

STRUCTURE HARVESTER suggested that the genotypic data best fit a two-population model (Figure 4.1); however, these results are based on the maximum difference in the log probability (ΔK) of each successive K. Each population (K_i) estimate takes into account information from the previous (K_{i-1}) and following population (K_{i+1}) estimate to calculate ΔK. Therefore, in order to calculate ΔK for $K = 1$, STRUCTURE HARVESTER would need information for $K = 0$, which does not exist. Consequently, there is no estimate of ΔK for $K = 1$ and it cannot be selected as the best model (Table 4.2). Similar values for mean Ln $P(K)$ for $K = 1$ and $K = 2$ populations suggests little improvement between models. Prichard et al. (2000) suggests researchers should be skeptical of inferred population structure in instances of small differences

Table 4.1 Summary Statistics for 307 Mottled Ducks (*Anas f. maculosa*) sampled from 12 locations in the western Gulf Coast including sample size (*n*), observed (H_O) and expected (H_E) heterozygosity, allelic richness (AR) for sampling locations with less than 10 individuals (n < 10) and sampling locations with 10 or more individuals (n ≥ 10), and inbreeding coefficient (F_{IS}).

Sampling location	*n*	H_O	H_E	AR (*n* < 10)	AR (*n* ≥ 10)	F_{IS}
Atchafalaya Delta WMA, LA	8	0.5403	0.6542	1.646	-	0.1583
Big Burns Marsh, LA	17	0.5268	0.6511	1.647	4.669	0.1765
Cameron-Prairie NWR, LA	10	0.5799	0.6494	1.645	4.761	0.1358
Caernarvon, LA	2	0.5694	0.6204	1.620	-	0.1183
J.D. Murphree WMA, TX	31	0.5752	0.6637	1.662	4.775	0.1183
Justin Hurst WMA, TX	75	0.5421	0.6652	1.664	4.768	0.1807
Mad Island WMA, TX	61	0.5717	0.6601	1.659	4.797	0.1313
Marsh Island SWR, LA	29	0.5321	0.6759	1.673	4.876	0.2078
Mobile-Tensaw Delta, AL	5	0.6556	0.6972	1.692	-	0.0600
Pass-a-Loutre WMA, LA	8	0.5678	0.6354	1.630	-	0.0991
Pointe aux Chenes WMA, LA	27	0.5261	0.6502	1.648	4.660	0.1833
Rockefeller SWR, LA	34	0.5440	0.6702	1.668	4.767	0.1834
Mean (± std. error)		0.5609 (± 0.0099)	0.6578 (± 0.0054)	1.654 (± 0.005)	4.759 (± 0.023)	0.1461 (± 0.0119)

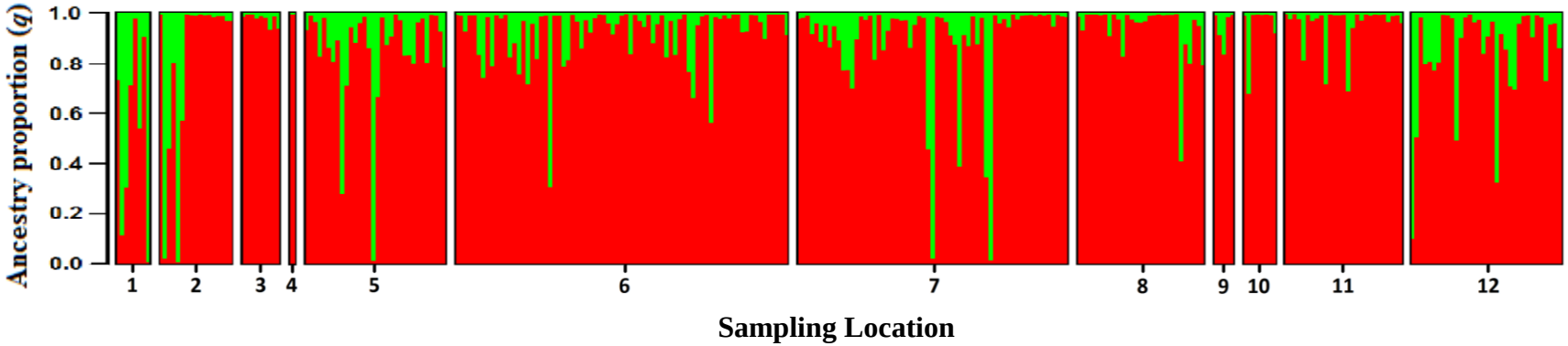


Figure 4.1 Population assignment bar graph from STRUCTURE based on 36 microsatellite loci for western Gulf Coast Mottled Ducks for 12 sampling locations. Sampling location abbreviations are as follows: Atchafalaya Delta WMA, LA (1), Big Burns Marsh, LA (2), Cameron-Prairie NWR, LA (3), Caernarvon, LA (4), J.D. Murphree WMA, TX (5), Justin Hurst WMA, TX (6), Mad Island WMA, TX (7), Marsh Island SWR, LA (8), Mobile-Tensaw Delta, AL (9), Pass-A-Loutre WMA, LA (10), Pointe aux Chenes WMA, LA (11), and Rockefeller SWR, LA (12).

Table 4.2 Log probability of data as a function of successive *K* values ranging from 1 – 14 for Mottled Ducks at 36 microsatellite loci using the admixture model implemented in the program STRUCTURE.

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	ΔK
1	10	-36971.25	0.5817	NA	NA	NA
2	10	-36293.89	9.7183	677.36	358.26	36.86446
3	10	-35974.79	7.2928	319.10	9.120	1.250542
4	10	-35664.81	7.0801	309.98	48.10	6.793699
5	10	-35402.93	8.5626	261.88	139.05	16.23925
6	10	-35280.10	17.9235	122.83	55.09	3.073611
7	10	-35102.18	17.3065	177.92	26.41	1.526016
8	10	-34950.67	39.1399	151.51	15.09	0.38554
9	10	-34814.25	43.2365	136.42	109.16	2.524719
10	10	-34786.99	172.9061	27.26	135.68	0.784703
11	10	-34624.05	26.0769	162.94	92.17	3.534551
12	10	-34553.28	35.6267	70.77	14.68	0.412050
13	10	-34467.83	36.2519	85.45	48.58	1.340068
14	10	-34430.96	42.4373	36.87	NA	NA

in *P* (K) if there is no clear biological explanation in the assignments. When mean *q* values were sorted (pop. 1 < *q* =0.5 < pop. 2) from the proposed best fit model (*K* = 2), no obvious geographic pattern emerged from the two populations (Table 4.3). Most individuals were assigned to one putative population (94.1%), whereas only 18 individuals were assigned to the other putative population (5.9%) and these individuals did not consistently originate from the same or nearby sampling locations. For example, if population structure really existed between sampling locations, it would be expected that all or most of the individuals from a location would be assigned to one genetic cluster. Instead, the cluster with 18 individuals draws individuals from multiple locations (Table 4.3).

The AMOVA population differentiation test among 12 Mottled Duck sampling locations estimated a global F_{ST} value of 0.0088, and was nonsignificant (*P* = 0.9926; Table 4.4), which

Table 4.3 Designation of individuals assigned to either Population 1 ($q < 0.5$) or Population 2 ($q > 0.5$) determined from mean q values for $K = 2$ populations as determined in the program STRUCTURE using the admixture model.

Sampling Location	*n*	Individuals assigned to Population 1 (%)	Individuals assigned to Population 2 (%)
Atchafalaya Delta WMA, LA	8	5 (62.5)	3 (37.5)
Big Burns Marsh, LA	17	14 (82.4)	3 (17.6)
Cameron-Prairie NWR, LA	9	9 (100.0)	0 (0.0)
Caernarvon, LA	2	2 (100.0)	0 (0.0)
J.D. Murphree WMA, TX	32	30 (93.8)	2 (6.3)
Justin Hurst WMA, TX	75	74 (98.7)	1 (1.3)
Mad Island WMA, TX	61	56 (91.8)	5 (8.2)
Marsh Island SWR, LA	29	28 (96.6)	1 (3.4)
Mobile-Tensaw Delta, AL	5	5 (100.0)	0 (0.0)
Pass-A-Loutre WMA, LA	8	8 (100.0)	0 (0.0)
Pointe aux Chenes WMA, LA	27	27 (100.0)	0 (0.0)
Rockefeller SWR, LA	34	31 (91.2)	3 (8.8)
Total (mean %)	307	289 (94.1)	18 (5.9)

Table 4.4 AMOVA results using microsatellite data from 12 western Gulf Coast Mottled Duck sampling locations.

Source of Variation	d.f.	Sums of Squares	Variance Components	Percentage of Variation
Among Populations	11	178.512	0.0937	0.88
Among Individuals within Populations	295	3467.244	1.1828	11.09
Within Individuals	307	2882.000	9.3876	88.03
Total	613	6527.756	10.6642	

suggests no population structure. Most of the genetic variation in microsatellite data (88.03%) was attributed to within-individual variation (Table 4.4). Pairwise estimates of F_{ST} for western

Gulf Coast Mottled Ducks showed small and significant differences for nearly half of the sampling location comparisons (31 out of 66), ranging from -0.0149 to 0.0603 (Table 4.5). *F*-statistics measure the deficit of heterozygotes against expected Hardy-Weinberg proportions in the examined population (Allendorf and Luikart 2007). Specifically, F_{ST} is a measurement of genetic divergence among subpopulations, or in this case, Mottled Duck sampling sites (assuming these represent true subpopulations). F_{ST} values range from zero to one, but can be negative if the mean expected heterozygosity over all subpopulations (H_S) is greater than the expected Hardy-Weinberg heterozygosity if the population were panmictic (H_T). R_{ST} estimates ranged from -0.0704 to 0.2766 (Table 4.5). Atchafalaya Delta WMA showed the highest mean pairwise F_{ST} (0.0423 ± 0.0033) and R_{ST} estimates (0.1944 ± 0.0121) of all sampling locations (F_{ST} = 0.0105 ± 0.0016, R_{ST} = 0.0018 ± 0.0035). The PCA plot for Mottled Ducks across 12 sampling locations indicates little structure. Axis one and two only explained 2.75% of the variation, and individuals grouped closely and showed no obvious separation by location (Figure 4.2).

Mean Bayesian estimates in the program MIGRATE-N (three regions model) revealed higher mutation-scaled effective population size in the eastern region than in the central or western regions. Most migration occurred from the western to the central region, and the least from the eastern region to the central region (Figure 4.3a; migration between western and eastern regions was not estimated). For the two regions model, mean Bayesian estimates revealed a higher mutation-scaled effective population size for eastern than western Mottled Ducks, and similar levels of migration between regions, although there tended to be more migration from the western to the eastern region (Figure 4.3b).

Table 4.5 Pairwise estimates of F_{ST} (below diagonal) and R_{ST} (above diagonal) for 12 sampling locations using 307 western Gulf Coast Mottled Ducks samples. Significant F_{ST} P-values ($P < 0.05$) indicated in bold.

	Sampling Location											
	AD_LA	BB_LA	CP_LA	CV_LA	JD_TX	JH_TX	M_TX	M_LA	M_AL	PL_LA	PC_LA	R_LA
AD_LA		0.1704	0.2143	0.1693	0.1992	0.1534	0.1719	0.2114	0.2766	0.1264	0.2414	0.2044
BB_LA	**0.0477**		-0.0141	-0.0192	0.0059	-0.0034	-0.0015	0.0125	0.0032	-0.0045	0.0579	0.0052
CP_LA	0.0405	-0.0101		-0.0704	-0.0111	-0.0069	-0.0130	-0.0184	-0.0524	0.0116	-0.0103	-0.0047
CV_LA	0.0582	0.0034	-0.0039		-0.0064	-0.0512	-0.0412	0.0040	-0.0307	0.0073	0.0131	0.0317
JD_TX	0.0384	0.0101	0.0082	-0.0046		0.0038	-0.0052	0.0022	-0.0170	0.0216	0.0283	0.0031
JH_TX	**0.0266**	**0.0150**	0.0074	-0.0088	**0.0093**		-0.0014	0.0135	-0.0012	0.0161	0.0468	0.0077
M_TX	**0.0422**	**0.0177**	0.0154	-0.0112	0.0026	**0.0075**		0.0001	-0.0181	0.0192	0.0262	0.0051
M_LA	**0.0382**	0.0052	-0.0027	-0.0149	0.0063	**0.0119**	**0.0146**		-0.0307	0.0265	0.0055	0.0075
M_AL	0.0289	0.0239	0.0188	0.0064	0.0006	0.0072	0.0013	0.0135		0.0395	-0.0062	-0.0271
PL_LA	**0.0603**	0.0077	0.0066	0.0226	**0.0325**	**0.0250**	**0.0306**	**0.0146**	**0.0402**		0.0634	0.0274
PC_LA	**0.0527**	**0.0148**	-0.0020	0.0060	**0.0287**	**0.0262**	**0.0304**	**0.0082**	0.0308	**0.0226**		0.0484
R_LA	**0.0311**	**0.0104**	0.0015	-0.0067	**0.0066**	**0.0077**	**0.0101**	**0.0073**	0.0023	**0.0228**	**0.0180**	

Sampling location abbreviations are as follows: Atchafalaya Delta WMA, LA (AD_LA), Big Burns Marsh, LA (BB_LA), Caernarvon, LA (CV_LA), Cameron-Prairie NWR, LA (CP_LA), J.D. Murphree WMA, TX (JD_TX), Justin Hurst WMA, TX (JH_TX), Marsh Island SWR, LA (M_LA), Mad Island WMA, TX (M_TX), Mobile-Tensaw Delta, AL (M_AL), Pointe aux Chenes WMA, LA (PC_LA), Pass-A-Loutre WMA, LA (PL_LA), and Rockefeller SWR, LA (R_LA).

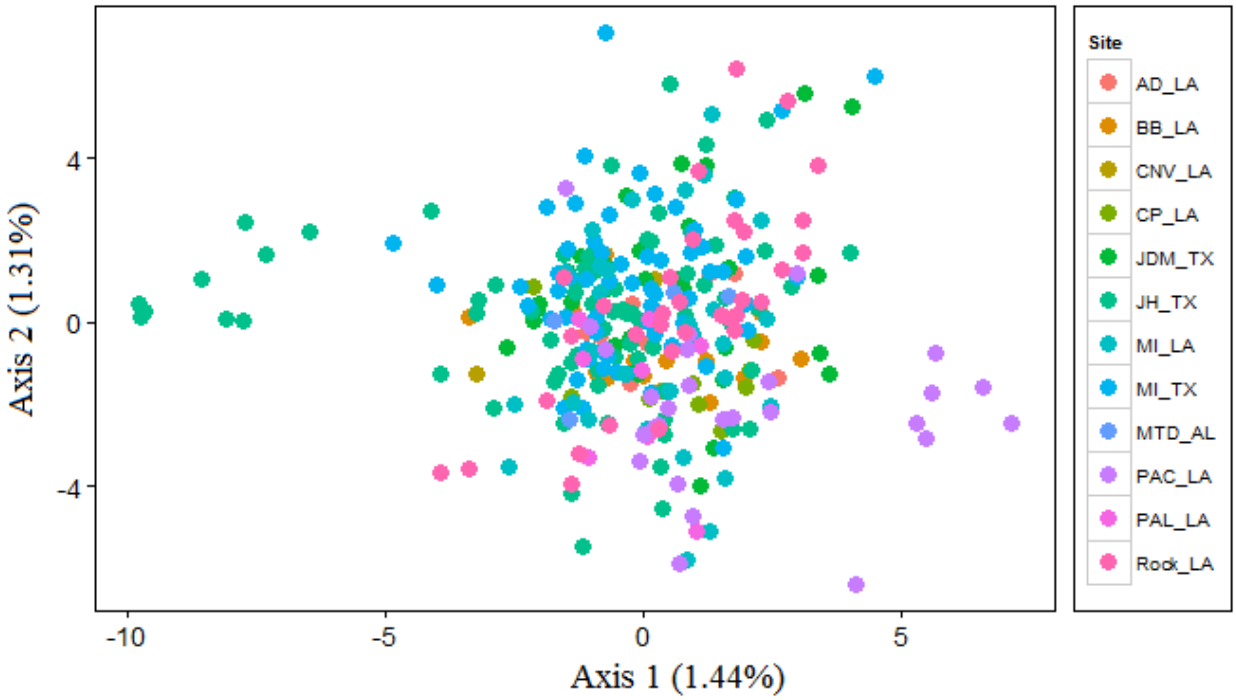
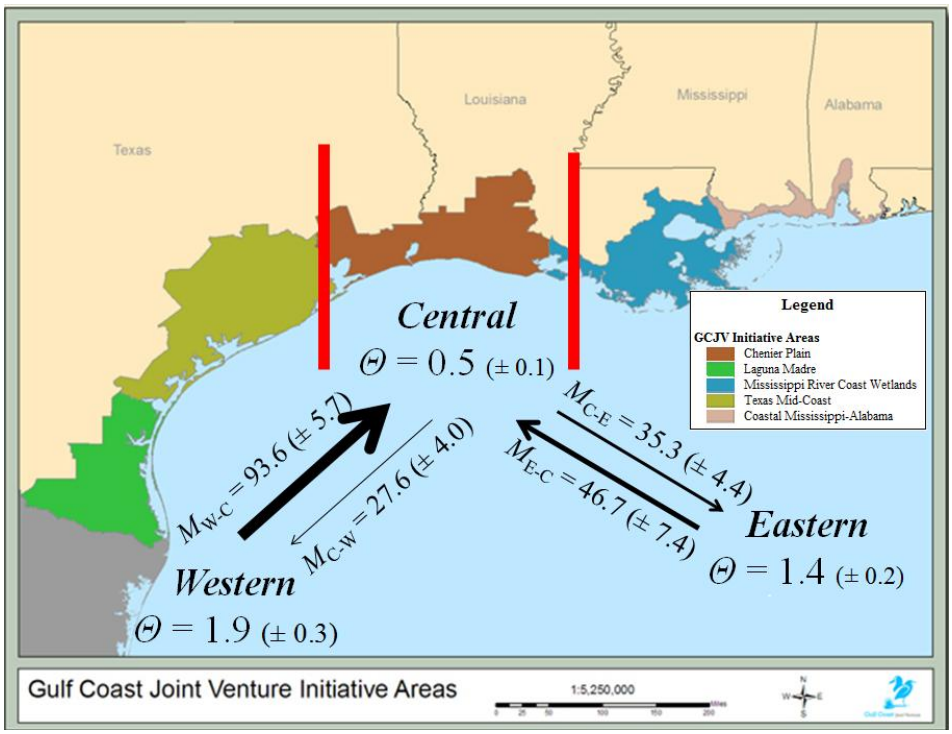
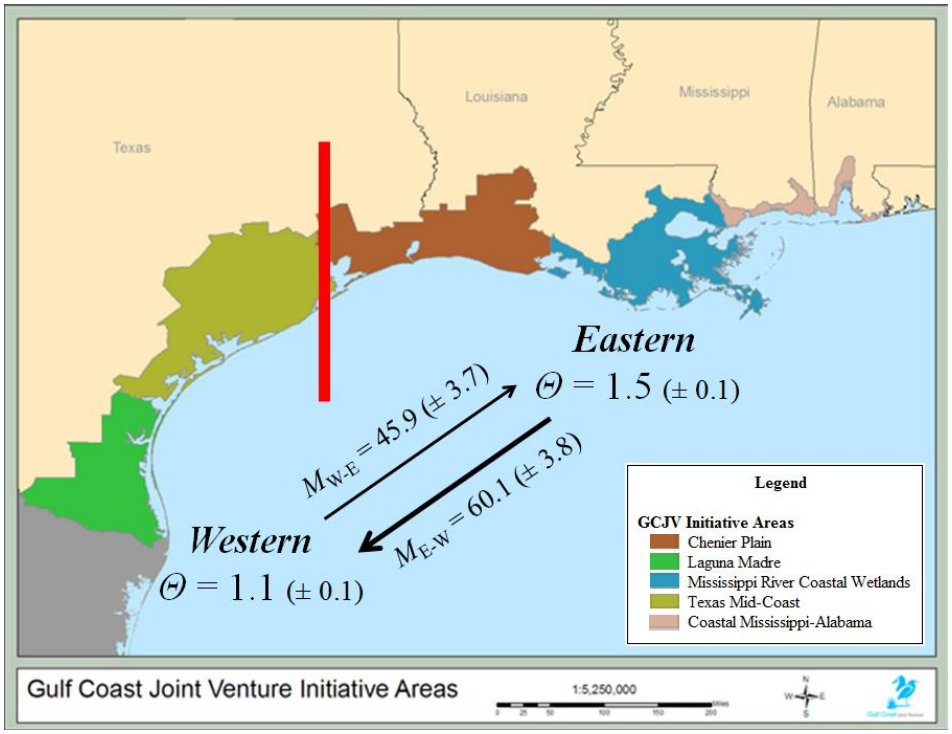


Figure 4.2 Principal Component Analysis plot conducted in program R using the heirfstat package for 12 western Gulf Coast Mottled Duck sampling locations. Sampling location abbreviations are as follows: Atchafalaya Delta WMA, LA (AD_LA), Big Burns Marsh, LA (BB_LA), Caernarvon, LA (CNV_LA), Cameron-Prairie NWR, LA (CP_LA), J.D. Murphree WMA, TX (JDM_TX), Justin Hurst WMA, TX (JH_TX), Marsh Island SWR, LA (MI_LA), Mad Island WMA, TX (MI_TX), Mobile-Tensaw Delta, AL (MTD_AL), Pointe aux Chenes WMA, LA (PAC_LA), Pass-A-Loutre WMA, LA (PAL_LA), and Rockefeller SWR, LA (Rock_LA).



a)



b)

Figure 4.3 Map of the western Gulf Coast showing a) Mottled Ducks split into three regions with estimates for mutation-scaled effective population size (mean θ ± std. error) and directional migration rates per generation (mean *M* ± std. error) for western, central, and eastern regions, and b) Mottled Ducks split into two regions with estimates for mutation-scaled effective population size (mean θ ± std. error) and directional migration rates per generation (mean *M* ± std. error) for a western region and an eastern regions. Maps adapted from the Gulf Coast Joint Venture website: www.gcjv.org/projects.php.

4.4 DISCUSSION

Mottled Ducks in the western Gulf Coast appear to consist of a single genetic population. Although STRUCTURE HARVESTER suggested two populations (K = 1 cannot be chosen), mean probability values, PCA analysis, and AMOVA results suggest that there is one genetic Mottled Duck population in the western Gulf Coast. One explanation for this might be inferred from band-recovery data from 1950 to 2010, which indicates that there is some dispersal across the western Gulf Coast. Of all the recoveries of Louisiana banded Mottled Ducks, 8.9% were in Texas (Baldassarre 2014). In Texas, 22.2% of banded Mottled Ducks were recovered in Louisiana (Baldassarre 2014). Western Gulf Coast Mottled Duck recoveries in states other than banded, coupled with ducks that are undetected (unbanded) and move between states, probably ensure that there is enough gene flow to produce one genetic population.

When western Gulf Coast Mottled Ducks were split into three regions according to habitat types and Gulf Coast Joint Venture initiative areas, most migration occurred from the western to the central region and similar migration rates occurred between the central and eastern regions. When western Gulf Coast Mottled Ducks were split into two regions, gene flow estimates were similar between the two regions; however, it was expected that significantly more migration would occur from the western region into the eastern, because coastal habitat in the western region along the central coast of Texas is thought to be inferior to the coastal habitat in the eastern region from the Chenier Plain through the Mississippi River delta system. My estimate of migration rates (two regions model; Figure 4.3b) do not reflect band-recovery data, perhaps because most of the recoveries in Texas and Louisiana are within the Chenier Plain Gulf Coast Joint Venture Initiative area, which is located in both states (W. Selman, LDWF, unpublished data; Figure 4.3). Therefore, it would be beneficial to analyze band-recovery data

based on habitats used by Mottled Ducks rather than the geographical border separating Texas and Louisiana in order to determine if genetic migration rate estimates agree with band-recovery data.

Pairwise F_{ST} and R_{ST} comparisons were consistent by sampling location, suggesting there is minimal genetic differentiation among western Gulf Coast Mottled Duck sampling locations. However, Atchafalaya Delta WMA seems to be the most divergent of all sampling locations, although the sample size in this study was low (n = 8). Peters et al. (2014) found similar results, where Mottled Ducks from Atchafalaya Delta WMA differed significantly from all other sampling localities in mtDNA. Likewise, McCracken et al. (2001) found the least amount of mtDNA haplotype and nucleotide diversity at Atchafalaya Delta WMA among all other sampling locations, and no radio-marked female Mottled Ducks on Atchafalaya Delta WMA made substantial movements from 2007 – 2009 (Davis 2012). Mottled Ducks at Atchafalaya Delta WMA may appear the most divergent in this study due to small sample sizes or high site fidelity.

The mean inbreeding coefficient (F_{IS}) across 12 Mottled Duck sampling locations 0.1461. Thus, individuals within sampling locations appear to be more inbred than what is expected for two individuals drawn at random from the entire western Gulf Coast population.

Mottled Ducks and Mexican Ducks are the only non-migratory dabbling ducks in North America, therefore, it would be interesting to compare genetic structure between these two species; unfortunately, the population structure of Mexican Ducks has not been studied. Population structure has been studied in other sedentary duck species including the New Zealand Blue Duck (*Hymenolaimus malacorhynchos*) and the Harlequin Duck (*Histrionicus histrionicus*). Endangered Blue Ducks endemic to New Zealand are riverine specialists (Robertson et al. 2007) and have highly fragmented populations on both the North and South Islands. Triggs *et al.*

(1992) found that Blue Ducks had high levels of genetic relatedness and inbreeding within populations using DNA fingerprinting, suggesting that dispersal is limited. A similar conclusion was reached by Robertson et al. (2007), who found strong and significant genetic structure both within and among the islands of New Zealand using mtDNA. Robertson et al. (2007) highlight that Blue Ducks show similar levels of genetic diversity to several non-threatened duck species, including the Mottled Duck (McCracken et al. 2001; Kulikova et al. 2005). Western Gulf Coast Mottled Ducks do not have populations that are highly fragmented analogous to New Zealand Blue Ducks, however, they show moderate inbreeding levels likely due to limited dispersal.

Harlequin Ducks in the nearshore environments of Alaska Peninsula/Kodiak Archipelago (APKA) and Prince William Sound (PWS) are year-round residents that have little or no spatial population structuring (Lanctot et al. 1999). The lack of genetic structuring in APKA and PWS Harlequin Ducks may be due to a recent range expansion, lack of barriers to gene flow, low but sufficient levels of emigrating juvenile birds between habitats, or dispersal due to habitat changes (Lanctot et al. 1999). It is possible that Mottled Ducks in the western Gulf Coast may share similar life-history attributes as Harlequin Ducks in the APKA and PWS, such as juvenile emigration and dispersal due to habitat changes. Band-recovery data has shown that more Mottled Ducks banded in Texas leave for Louisiana, possibly due to habitat changes in Texas during the year. Additionally, Stutzenbaker (1988) noted that juvenile dispersal of Mottled Ducks may sustain the connectivity between western Gulf Coast populations.

In this study, western Gulf Coast Mottled Ducks showed similar levels of genetic diversity across sampling locations and comparable levels of genetic diversity to North American Mallards, a finding consistent with Peters et al. (2014). Peters et al. (2014) hypothesized that

limited historical gene flow between Mottled Ducks and Mallards could explain high genetic diversity in western Gulf Coast Mottled Ducks.

Although two subspecies of Mottled Ducks demonstrate genetic structure at a broad geographical scale (McCracken et al. 2001; Williams et al. 2005b, Baldassarre 2014), additional structure at finer scales does not appear to exist in the western Gulf Coast. Genetic analysis of Mottled Ducks in the western Gulf Coast suggests that they can be managed regionally, rather than on a local scale.

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CHAPTER 5. CONCLUSION

The Mottled Duck is a non-migratory dabbling duck with populations in Florida and the western Gulf Coast. Despite concerns of hybridization between Mallards and Mottled Ducks, hybridization rates in the western Gulf Coast were low and there was no obvious geographic pattern in the distribution of hybrids.

An identification key created by Bielefeld et al. (in review) to distinguish Florida Mottled Ducks from Mallards and their hybrids proved extremely effective for the western Gulf Coast population. The key will provide standardized Mottled Duck versus Mallard and hybrid feather characteristics that will allow mangers to obtain correct identifications in the field should they wish to cull Mallards or hybrids in order to prevent future hybridization.

Data on Mottled Duck genetic structure indicates that there is only one genetic population in the western Gulf Coast; therefore, Mottled Duck populations can be managed regionally. The connectivity of Mottled Ducks in the western Gulf Coast is probably due to sufficient dispersal. Accordingly, these populations are unlikely to lose much genetic variation through genetic drift or to be strongly affected by inbreeding depression.

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APPENDIX 1. MICROSATELLITE LOCI CHARACTERISTICS

Characteristics of 36 microsatellite loci used in this study to genotype 405 ducks including Mottled Ducks, Mallards, and hybrids. Information is given for optimized primers including: amount of primer (P), magnesium chloride ($MgCl_2$), betaine, annealing temperature in degrees Celsius (T_A), and number of cycles (C). Each primer was described in Seyoum *et al.* 2012.

Locus	P (µM)	$MgCl_2$ (mM)	betaine (M)	T_A (°C)	C	Locus	P (µM)	$MgCl_2$ (mM)	betaine (M)	T_A (°C)	C
Aful04	1.0	1.50	0.2	59	35	Aful38	1.0	1.50	0.8	50	35
Aful05	1.0	1.00	0.8	58	35	Aful39	0.7	1.50	0.2	50	40
Aful07	1.0	1.50	0.2	50	35	Aful41	0.8	1.50	0.2	50	40
Aful08	1.0	1.50	0.8	50	35	Aful43	1.0	1.50	0.8	50	35
Aful10	1.0	1.50	0.8	54	35	Aful44	1.0	1.50	0.8	50	35
Aful14	1.0	1.50	0.8	50	35	Aful46	1.0	1.00	0.8	54	35
Aful17	0.8	1.25	0.2	54	35	Aful49	1.0	1.50	0.8	50	35
Aful19	1.0	1.25	0.8	50	35	Aful51	1.0	1.25	0.2	55	35
Aful20	1.0	1.25	0.8	50	40	Aful55	1.0	1.25	0.8	62	35
Aful25	1.0	1.25	0.8	50	40	Aful56	1.0	1.50	0.8	50	35
Aful28	1.0	1.50	0.8	50	35	Aful57	1.0	1.50	0.8	63	35
Aful29	1.0	1.25	0.8	50	35	Aful58	0.8	1.50	0.2	51	40
Aful30	1.0	1.25	0.8	50	35	Aful61	1.0	1.25	0.8	56	35
Aful31	1.0	1.50	0.8	61	35	Aful62	1.0	1.25	0.2	48	35
Aful33	1.0	1.50	0.2	50	35	Aful64	1.0	1.50	0.2	56	35
Aful34	1.0	1.25	0.8	52	45	Aful69	1.0	1.50	0.2	51	35
Aful35	1.0	1.50	0.8	61	35	Aful81	1.0	1.50	0.8	56	35
Aful37	1.0	1.50	0.8	62	35	Aful87	0.5	1.25	0.2	56	40

APPENDIX 2. MITOCHONDRIAL LOCI CHARACTERISTICS

Primers used to amplify and sequence mitochondrial genes cytochrome b (*Cyt b*) and NADH dehydrogenase subunit 2 (*ND2*). Numbers indicate the location of the 3' base in the light (L) and heavy (H) strand of the mitochondrial genome of *Gallus gallus* (Desjardins and Morais 1990)

Name	Sequence	Reference
Cyt b		
L14990	5' – AACATCTCCGCAATGATGAAA – 3'	Johnson and Sorenson (1998)
H16064	5' – CTTCGATTTTTGGTTTACAAGACC – 3'	Johnson and Sorenson (1998)
ND2		
L5219	5' – CCCATACCCCGAAAATGATG – 3'	Johnson and Sorenson (1998)
H6031	5' – CACTTTGGTATAAACCCTGT – 3'	Donne-Gousse *et al.* (2002)

APPENDIX 3. HARDY-WEINBERG EQUILIBRIUM ANALYSIS BY SAMPLING LOCATION

P-values for Hardy-Weinberg equilibrium analysis in GENEPOP for 36 microsatellite loci at 12 sampling locations for western Gulf Coast Mottled Ducks. Bold type indicates *P*-values are significant at $p < 0.05$.

	Sampling Location											
Locus	AD_LA	BB_LA	CP_LA	CV_LA	JD_TX	JH_TX	M_TX	M_LA	MTD_AL	PAL_LA	PAC_LA	R_LA
Aful04	NA	1	1	0.3333	0.4013	0.0583	**0.0206**	0.5324	0.6952	NA	**0.0065**	0.8294
Aful05	0.6595	0.229	0.8166	1	0.1523	0.1431	0.1344	0.2517	0.8454	0.936	**0.0307**	0.0686
Aful07	0.4406	0.3616	0.319	0.3333	**0.0124**	**0.0001**	**0.0014**	**0**	**0.0095**	0.0676	**0.0059**	0
Aful08	0.2136	**0.0058**	0.5367	NA	**0.0168**	**0**	**0.0003**	**0**	0.6211	0.1128	**0.0002**	**0.0001**
Aful10	1	0.523	NA	NA	1	1	1	0.4399	NA	NA	NA	1
Aful14	**0.0276**	0.2608	0.7236	NA	0.98	**0.0404**	0.609	0.5546	0.6952	0.7016	0.1237	0.3775
Aful17	0.376	0.4789	0.1945	1	0.6894	0.1692	**0.0131**	0.9308	0.7911	0.8473	0.5647	0.7659
Aful19	0.9108	0.6563	0.2569	1	**0.0357**	0.1491	**0.024**	0.0532	**0.0465**	0.4885	0.0778	**0.0607**
Aful20	0.3846	1	1	NA	**0.0002**	**0.0019**	0.0548	0.4041	0.619	0.269	**0.0081**	**0.0009**
Aful25	1	0.607	NA	0.3333	1	0.1553	0.5062	0.4702	1	0.7762	0.4286	0.1544
Aful28	0.3562	**0.0006**	0.4342	1	0.2326	**0.0011**	0.1438	0.3977	0.7714	0.8693	0.8018	**0.0006**
Aful29	0.9138	0.8775	0.0611	1	0.6586	**0.0092**	0.3344	0.9523	0.2852	0.4208	0.1273	**0.0118**
Aful30	0.0594	0.1054	0.7147	NA	0.2028	**0**	**0.0022**	**0**	1	**0.0053**	**0**	**0**
Aful31	0.1175	0.1983	0.7642	0.3333	0.5073	0.6354	0.0941	0.4608	1	0.3145	0.2971	**0.0122**
Aful33	1	**0.0108**	1	NA	0.151	**0**	0.2526	**0.0044**	1	1	0.3265	**0.0115**
Aful34	0.1113	**0.0121**	0.1802	1	0.5696	**0.0173**	0.7486	0.1409	1	0.3802	0.2159	**0.0051**
Aful35	0.8979	0.4064	0.2409	NA	0.1222	0.4436	0.9395	0.2052	1	0.2864	0.8328	0.1346
Aful37	1	**0.0098**	0.5946	1	0.6193	0.1451	0.2577	0.3043	0.9005	0.5524	0.1594	0.7211
Aful38	0.2727	0.2826	1	NA	**0.0002**	**0.0176**	**0.0024**	0.4044	0.6952	0.4126	**0.0428**	0.1477
Aful39	**0.0117**	**0.0002**	**0.021**	NA	0.8482	**0.001**	**0.0208**	**0.0096**	0.6131	**0.007**	**0**	**0.0074**
Aful41	0.5301	0.4577	0.4701	NA	0.6755	0.184	0.1612	0.6377	0.1873	0.0862	1	0.2091
Aful43	0.7636	0.5956	0.4563	1	0.2762	**0.041**	**0.0019**	**0.0007**	1	0.0751	**0.0381**	0.132
Aful44	0.1429	**0.0014**	0	1	**0.0009**	**0**	**0**	**0**	0.6577	**0.0043**	**0**	**0**
Aful46	0.3333	**0.0334**	1	NA	0.3929	**0.0093**	0.1335	**0.0293**	1	1	**0.0006**	**0.0003**
Aful49	NA	**0.0005**	0.0667	NA	**0**	**0**	**0**	**0**	0.6592	**0**	**0**	**0**

(Appendix 3 continued)

	Sampling Location											
Locus	AD_LA	BB_LA	CP_LA	CV_LA	JD_TX	JH_TX	M_TX	M_LA	MTD_AL	PAL_LA	PAC_LA	R_LA
Aful51	1	0.4547	1	1	0.8344	0.1735	0.4942	**0.0072**	1	0.8763	**0.0219**	0.1958
Aful55	1	0.1316	0.2757	0.3333	**0**	**0.0033**	0.1241	**0.0118**	0.0825	**0.0278**	0.2642	0.0879
Aful56	0.079	0.1499	0.9275	1	**0.017**	**0**	**0.0475**	0.1298	**0.0217**	0.5762	0.5092	**0.0238**
Aful57	NA	NA	1	NA	0.3065	1	1	1	NA	NA	1	0.2198
Aful58	0.0769	0.3791	0.2898	0.3333	0.8106	**0**	0.1239	0.194	1	0.2384	**0.0182**	0.0554
Aful61	0.3143	0.1476	**0.0008**	1	0.1123	**0.044**	**0**	0.1157	0.1515	0.7678	**0.0445**	0.1011
Aful62	**0.0397**	0.3169	1	1	0.4409	0.3505	0.7437	0.8103	1	1	**0.0034**	**0.0129**
Aful64	0.4406	**0.025**	**0.0412**	NA	0.2423	0.5342	**0.0361**	**0**	0.619	0.4805	**0**	0.2975
Aful69	0.1111	1	NA	NA	NA	1	1	NA	NA	1	1	1
Aful81	0.031	0.2189	1	0.3333	0.481	**0**	0.0689	**0.0254**	0.619	1	0.3014	1
Aful87	0.0909	0.1279	**0.0111**	NA	0.2463	**0**	**0.0002**	**0.0026**	1	0.4406	**0.0069**	**0**

Sampling location abbreviations are as follows: Atchafalaya Delta WMA, LA (AD_LA), Big Burns Marsh, LA (BB_LA), Caernarvon, LA (CV_LA), Cameron-Prairie NWR, LA (CP_LA), J.D. Murphree WMA, TX (JD_TX), Justin Hurst WMA, TX (JH_TX), Marsh Island SWR, LA (M_LA), Mad Island WMA, TX (M_TX), Mobile-Tensaw Delta, AL (MTD_AL), Pointe aux Chenes WMA, LA (PAC_LA), Pass-A-Loutre WMA, LA (PAL_LA), and Rockefeller SWR, LA (R_LA).

APPENDIX 4. MALE MORPHOLOGICAL COMPARSION

Comparison of morphological characteristics for genetically and key assigned male Mottled Ducks, Mallards, and hybrids: a) Mallard in pre-alternate molt (top), hybrid (middle), and Mottled Duck (bottom), b) Mallard in pre-alternate molt (left), hybrid (middle), and Mottled Duck (right), and c) Mallard in pre-alternate molt (left), hybrid (middle), and Mottled Duck (right).

a)

b)

c)

APPENDIX 5. FEMALE MORPHOLOGICAL COMPARSION

Comparison of morphological characteristics for genetically and key assigned female Mottled Ducks, Mallards, and hybrids: a) Mallard in pre-alternate molt (top), hybrid (middle), and Mottled Duck (bottom), b) Mallard in pre-alternate molt (left), hybrid (middle), and Mottled Duck (right), and c) Mallard in pre-alternate molt (left), hybrid (middle), and Mottled Duck (right).

a)

b)

c)

VITA

Robert Ford was raised in Mt. Morris, Michigan, and received his bachelor's degree at Michigan State University (MSU) in 2012. While at MSU, he worked for the U.S. Forest Service (USFS) as a biological science technician in Oscoda, Michigan, and at the Aquatic Animal Health Laboratory (AAHL) on the campus of MSU as a research assistant. With the education obtained from MSU and professional work experience gained while working with the USFS and AAHL, he entered graduate school in the School of Renewable Natural Resources at Louisiana State University. He is a candidate to receive his master's degree in December 2015 and plans to pursue work with a state, federal, or private wildlife management agency upon graduation.