Hybridization between Mottled Ducks (Anas fulvigula maculosa) and Mallards (A. platyrhynchos) in the western Gulf Coast region

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**RESEARCH ARTICLE**

Hybridization between Mottled Ducks (*Anas fulvigula maculosa*) and Mallards (*Anas platyrhynchos*) in the western Gulf Coast region

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**ABSTRACT**

Hybridization between species that do not normally interbreed has increased due to human impacts on natural environments, such as habitat alteration or introductions of nonnative species. In particular, the introduction of Mallards (*Anas platyrhynchos*) globally has led to hybridization with many duck species. In the southeastern United States, hybridization with Mallards is a potential threat to the genetic identity of Mottled Ducks (*A. fulvigula*), a nonmigratory, coastal duck species. Hybridization between Mallards and Mottled Ducks has been examined in South Carolina and Florida, but not extensively in the remaining part of the Mottled Duck range. Mottled Ducks introduced into South Carolina show considerable admixture with Mallards, whereas in Florida, the hybridization rate between Mallards and Mottled Ducks is ~9%. Given these results, hybridization in the western Gulf Coast region is of potential concern and should be examined to determine the potential impact on the Mottled Duck lineage. In this study, we examined hybridization in the western Gulf Coast region using 36 microsatellite loci and 405 ducks consisting of putative Mottled Ducks (n = 319), Mallards (n = 76), and hybrids (n = 10). Overall, genetic analyses revealed levels of hybridization of ~5–8%, with more individuals genetically assigned as hybrids from birds putatively identified as Mallards (5/76 and 14/76 in STRUCTURE and INSTRUCT, respectively) than as Mottled Ducks (14/319 in both programs). These data suggest that hybridization rates in the western Gulf Coast region are lower than those in Florida. However, projected coastal marsh loss in Texas and Louisiana may drive Mottled Ducks into urban wetland areas, an outcome that may increase contact between Mottled Ducks and Mallards and could result in similar levels of hybridization to those observed in Florida, where Mottled Ducks occupy urban and suburban habitats. Regular monitoring will help to determine future trends in levels of hybridization.

**Keywords:** hybridization, Mottled Duck, *Anas fulvigula maculosa*, Mallard, western Gulf Coast, waterfowl, conservation genetics

**Hibridación entre Anas fulvigula maculosa y A. platyrhynchos en el oeste de la Costa del Golfo**

La hibridación entre especies que normalmente no se entrecruzan ha aumentado debido a los impactos humanos en los ambientes naturales, como la alteración del hábitat o las introducciones de especies no nativas. En particular, la introducción de *Anas platyrhynchos* en todo el mundo ha generado la hibridación en muchas especies de patos. En el sudeste de Estados Unidos, la hibridación con *A. platyrhynchos* es una amenaza potencial a la identidad genética de *A. fulvigula*, una especie costera no migratoria de pato. La hibridación entre *A. platyrhynchos* y *A. fulvigula* ha sido examinada en Carolina del Sur y Florida pero no de modo extensivo en el resto del rango de *A. fulvigula*. La introducción de *A. fulvigula* en Carolina del Sur muestra una mezcla considerable con *A. platyrhynchos*, mientras que en Florida, las tasas de hibridación entre *A. platyrhynchos* y *A. fulvigula* son ~9%. Dados estos resultados, la hibridación en el oeste de la Costa del Golfo es una preocupación potencial y debería ser examinada para determinar el impacto potencial en el linaje de *A. fulvigula*. En este estudio, examinamos la hibridación en el oeste de la Costa del Golfo con 36 microsatélites y 405 individuos putativos de *A. fulvigula* (n = 319), *A. platyrhynchos* (n = 76) e híbridos (n = 10). En general, los análisis genéticos revelaron niveles de hibridación de ~5–8%, con más individuos genéticamente asignados como híbridos a partir de aves identificadas como individuos putativos de *A. platyrhynchos* (5/76 y 14/76 en STRUCTURE e INSTRUCT, respectivamente) que individuos putativos de *A. fulvigula* (14 de 319 en ambos programas). Estos datos sugieren que las tasas de hibridación en el oeste de la Costa del Golfo son más bajas que las de Florida. Sin embargo, la pérdida proyectada de marismas costeras en Texas y Luisiana podrían desplazar a *A. fulvigula* a áreas de humedales urbanos, un resultado que podría aumentar el contacto entre *A. fulvigula* y *A. platyrhynchos* dando como resultado niveles similares de hibridación, comparado con aquellos observados en Florida donde *A. fulvigula* ocupa...
Hybridization, or the interbreeding of individuals from genetically distinct populations (Rhymer and Simberloff 1996, Allendorf et al. 2001), plays an important role in the evolution of plant and animal species because organisms can acquire favorable alleles and novel gene combinations (Seehausen 2004, Allendorf and Luikart 2007, Rheindt and Edwards 2011). However, hybridization can also lead to the loss of genetically distinct lineages through introgressive hybridization: the incorporation of genes from one species into another when viable hybrids backcross with individuals from parental populations (Allendorf et al. 2001). Human-induced hybridization has increased in recent years, primarily from modifications to natural environments and deliberate or accidental species introductions. In this context, hybridization often threatens the genetic integrity of a variety of distinct species. For example, in passerines, habitat change appears to have allowed Blue-winged Warblers (Vermivora cyanoptera) to colonize areas occupied by Golden-winged Warblers (V. chrysoptera; Vallender et al. 2007, Crawford et al. 2017). Consequently, introgression of Blue-winged Warbler genes has occurred in a tree area of a phenotypically representative Golden-winged Warbler genomes (Vallender et al. 2007). Similarly, in mammals, the introduction of domestic caribou (Rangifer tarandus tarandus) into Alaska, USA, has resulted in moderate levels of hybridization with native caribou (R. t. granti) on Alaska’s North Slope (Mager et al. 2013). Finally, in fish, rainbow trout (Oncorhynchus mykiss) introduced into the upper Columbia River system in Idaho, USA, and British Columbia, Canada, now hybridize with populations of native westslope cutthroat trout (O. clarki lewisi), threatening their taxonomic integrity (Rubidge and Taylor 2004).

Hybridization between avian species is relatively common, but is most prevalent in Anseriformes (ducks, swans, and geese), members of which show the highest tendency to hybridize among birds (Grant and Grant 1992, McCarthy 2006). One member of the Anseriformes that frequently hybridizes, especially when introduced into 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 have been documented to hybridize with numerous other Anas species globally, in both their native range as well as in regions where they have been introduced. For example, the introduction of Mallards into New Zealand has most likely eliminated the native Pacific Black Duck (A. superciliosa superciliosa, known locally as the Grey Duck) lineage due to introgressive hybridization (Rhymer et al. 1994).

Mallards have become a significant conservation problem in North America because interbreeding between Mallards and all North American species within the Mallard complex (Mexican Duck [A. f. diazi], American Black Duck [A. rubripes], and Mottled Duck [A. fulvigula]) results in viable and fertile offspring (Brodsky and Weatherhead 1984, Williams et al. 2005). Increased levels of hybridization between Mallards and American Black Ducks appear to have been caused by habitat alteration, particularly during the 1960s and 1970s. As habitat changed, Mallards expanded their range eastward, colonizing areas previously dominated by American Black Ducks (Mank et al. 2004). Several decades of introgressive hybridization between American Black Ducks and newly arrived Mallards appear to have eroded genetic differentiation between the 2 species (Mank et al. 2004): $G_{ST}$, a measure of genetic divergence between populations, has decreased substantially from 0.1460 in American Black Duck–Mallard museum samples collected prior to 1940 to 0.0008 for samples collected in 1998 (Mank et al. 2004). Another member of the Mallard complex, the Mexican Duck, may be genetically similar to Mallards as a consequence of historical hybridization (McCracken et al. 2001). Furthermore, differentiation among Mexican Duck subpopulations may be caused in part by introgression with Mallards in the northern part of their range (Lavretsky et al. 2015). 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 nonmigratory, coastal dabbling duck species with native populations in peninsular Florida (A. fulvivula fulvivula) and the western Gulf Coast region from Alabama to northern Mexico (A. f. maculosa). In Florida, nonnative Mallards have increasingly invaded Mottled Duck breeding grounds as the human population of peninsular Florida and concomitant releases of farm-reared Mallards have increased (Williams et al. 2005). Approximately 12,000 domesticated Mallards have been released annually throughout Florida since the early 1990s, as estimated from farm and feed store sales (Bielefeld et al. 2010, R. Bielefeld personal communication), with hybridization recently estimated at ~9% (Williams et al. 2005). Introgression has also been reported in the introduced

**Palabras clave:** Anas fulvigula maculosa, Anas platyrhynchos, aves acuáticas, oeste de la Costa del Golfo, genética de la conservación, hibridación
South Carolina–Georgia population (South Atlantic Coastal Zone [SACZ] population; Peters et al. 2016); Mottled Ducks were translocated from Texas, Louisiana, and Florida to South Carolina in 1976 and 1982 (Weng 2006). Williams et al. (2005) reported a Mallard–Mottled Duck hybrid swarm in this population. Peters et al. (2016) excluded potential hybrids from their analyses of genetic differentiation between populations of Mottled Ducks and Mallards (based on plumage), but nevertheless observed considerable admixture between the 2 species. Although the introduced SACZ population represents an artificial situation, it demonstrates that high levels of hybridization can occur between these 2 closely related species.

In the western Gulf Coast region, research on hybridization has been identified as a priority action by the Gulf Coast Joint Venture Mottled Duck Conservation Plan (Wilson 2007) for several reasons: (1) Mottled Ducks are a species of conservation concern (Lester et al. 2005); (2) Mallards are known to frequent marinas and ponds near rural dwellings during the breeding season, where they may encounter and mate with Mottled Ducks (B. Wilson personal communication); (3) regulated releases of Mallards have historically occurred within the western Gulf Coast Mottled Duck range (B. Wilson personal communication); (4) interbreeding with Mallards could produce hybrids that are as fit as or more fit than Mottled Ducks (heterosis; Allendorf and Luikart 2007); and, finally, (5) little information exists on the level of hybridization between Mottled Ducks and Mallards.

Recent genetic work on Mottled Ducks has shown clear separation between Florida Mottled Ducks, western Gulf Coast–SACZ Mottled Ducks, and Mallards based on STRUCTURE and PCA analyses, with as much differentiation between Florida and western Gulf Coast Mottled Ducks as between Mottled Ducks and Mallards (Peters et al. 2016). In an earlier study, Peters et al. (2014) also attempted to genetically distinguish Mottled Ducks and Mallards and to identify putative hybrids using sequence data for 1 mitochondrial and 5 nuclear genes. Peters et al. (2014) concluded that a very small panel of loci was sufficient for distinguishing Mottled Ducks from Mallards, but provided low power for dependably detecting hybrids. Identifying hybrids typically requires numerous loci, and, for closely related species, 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 is needed in the western Gulf Coast region to determine levels of introgression. In this study, our goal was to estimate levels of hybridization between Mottled Ducks and Mallards in the western Gulf Coast region using a large panel of microsatellite markers. Results from this study can provide managers with an estimate of the percentage of hybrids in the western Gulf Coast region, which can be compared with estimates obtained in Florida, used to inform management (e.g., culling) of Mallards outside the winter residency period, and used as a baseline value to determine whether hybridization is increasing or decreasing in the future.

METHODS

Sampling

Putative Mottled Ducks were caught during the summer molt (June–August) in coastal Louisiana from 2011 to 2014 in conjunction with Louisiana Department of Wildlife and Fisheries (LDWF) banding operations. Upon capture, blood was drawn from each duck by brachial vein puncture and stored in Queen's lysis buffer (Seutin et al. 1991), and ducks were released safely afterward. 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 during 2009–2014 from locations spanning Texas, Louisiana, and Alabama (Figure 1, Table 1, Supplemental Material Table S1). Mallard tissue samples were obtained from Louisiana, Mississippi, and Alabama (Figure 1, Table 1, Supplemental Material Table S1). Ducks were classified to species by the collector (i.e., we initially assumed that the species assignment upon collection was correct).

Microsatellite Genotyping

DNA was extracted from putative Mottled Ducks (n = 319), Mallards (n = 76), and hybrids (n = 10) using Qiagen DNeasy Kits (Qiagen, Valencia, California, USA) and screened for amplification and polymorphism with 36 microsatellite loci developed for Mottled Ducks (Seyoum et al. 2012, Supplemental Material Table S2). All forward primers were labeled at the 5’ end with an M13-tail (5’-CAGCAGTTGTAAAGCAGC-3’) to allow detection of alleles. Polymerase chain reaction (PCR) amplifications contained 20 ng DNA, 1× standard Taq (Mg-free) reaction buffer (New England BioLabs, Ipswich, Massachusetts, USA), 0.8 mM dNTPs (Qiagen), 0.2 μL v/v 100% dimethyl sulfoxide (DMSO), 0.5 units Taq polymerase (New England BioLabs), 0.5–1.0 μM of each primer, 0.75–1.50 mM MgCl2 (New England BioLabs), 0.2–0.8 M betaine, 0.1–0.3 μM 5’ fluorescently labeled M13 forward primer (6FAM, NED, PET, or VIC; Applied Biosystems, Foster City, California, USA), and nanopure water for a final reaction volume of 10 μL (Supplemental Material Table S2). Reactions were carried out on Mastercycler proS (Eppendorf, Hamburg, Germany) and MyCycler (Bio-Rad Laboratories, Hercules, California, USA) thermal cyclers with the following conditions: 2 min at 95°C for initial denaturation, followed by 35–45 cycles of 30 s at 94°C, 30 s at the annealing temperature (Supplemental Material Table S2).
Table S2), and 30 s at 72°C for elongation, ending with a final elongation step of 72°C for 5 min.

Following optimization, all samples were sent to the Yale University DNA Analysis Facility on Science Hill (New Haven, Connecticut, USA) 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 in all fragment analyses to ensure consistent scoring of alleles. Genotypes were assigned using GENEMARKER 2.4.0 (Soft Genetics, State College, Pennsylvania, USA).

**Genetic Diversity**

Microsatellite loci were first checked in MICROCHECKER (van Oosterhout et al. 2004) for PCR artifacts and/or null alleles. Measures of genetic diversity were estimated separately by species after removing ducks inferred to be hybrids based on genetic admixture analysis. Genetic diversity was measured for each species as allelic richness (AR), observed heterozygosity ($H_O$), and expected heterozygosity ($H_E$) using the HIERFSTAT package (Goudet 2005) in R 3.1.2 (R Core Team 2014). Exact tests for departures from Hardy-Weinberg expectations for each locus and linkage disequilibrium for each locus pair in each population were calculated with GENEPOP 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, where significant estimates were based on 95% confidence intervals and those bracketing...
Genetic Mixture

Hybridization between Mottled Ducks and Mallards was inferred using programs STRUCTURE 2.3.4 (Pritchard et al. 2000) and INSTRUCT (Gao et al. 2007). STRUCTURE uses multilocus 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 (Gao et al. 2007). INSTRUCT is an alternative to STRUCTURE that does not assume Hardy-Weinberg equilibrium.

In STRUCTURE, models ranging from a single-population model to a 4-population model (K = 1–4) were tested using 10 replications for each model, with 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 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).

We used the proportion of each individual's ancestry (q) to assign each individual 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 that species, whereas individuals with <90% assigned ancestry were considered to be hybrids. Finally, output from STRUCTURE was used in program CLUM-
PAK (Kopelman et al. 2015) to create bar graphs for individuals according to their ancestry proportion (q) by population.

In INSTRUCT, models ranging from a single-population model to a 4-population model (K = 1–4) were tested using 5 chains for each model, with a burn-in of 200,000 steps followed by 500,000 MCMC iterations. Mode 1 was used to infer population structure with admixture. We used the proportion of each individual’s ancestry (q) to assign each individual 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 that species, whereas individuals with <90% assigned ancestry were considered to be hybrids. Estimates provided in the results section are means ± standard errors.

RESULTS

Genetic Diversity

MICROCHECKER indicated that 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 (STRUCTURE analysis) or 10 loci (INSTRUCT analysis; Supplemental Material Tables S3 and S4). Single locus deviations from Hardy-Weinberg expectations may have been the result of low sample sizes at some locations (e.g., Atchafalaya Delta WMA [n = 8], Caernarvon [n = 2], Mobile-Tensaw Delta [n = 5], and Pass-a-Loutre WMA [n = 8]), null alleles (Seyoum et al. 2012), and/or localized inbreeding. Localized inbreeding may occur because adult Mottled Ducks tend to be philopatric, and banded individuals are usually recovered in the same county in which they were banded (Stutzenbaker 1988). Additionally, global (all loci combined) deviations from Hardy-Weinberg equilibrium by population could have resulted from the influence of a single locus or a small number of loci with extremely significant departures from Hardy-Weinberg expectations (P < 0.001) in certain sampling locations.

After removing genetic hybrids identified by STRUCTURE, allelic richness for Mottled Ducks was 10.093 ± 1.039, while observed and expected heterozygosities were 0.5609 ± 0.0383 and 0.6620 ± 0.0348, respectively. Allelic richness for Mallards was 10.346 ± 0.937, while observed and expected heterozygosities were 0.5845 ± 0.0341 and 0.7086 ± 0.0350, respectively. Mottled Ducks showed linkage disequilibrium (LD) in a global test (all sampling sites) for ~5% (29 out of 630) of Bonferroni-corrected pairwise comparisons among loci, whereas Mallards showed linkage disequilibrium for <1% (1 out of 630) of pairwise comparisons among loci. Locus pairs that showed LD (~5%) in the global test for Mottled Ducks were in LD for only a few sampling locations (<4 loci; not Bonferroni corrected). One pair of loci (Aful08 and Aful30) showed LD in 5 out of 12 sampling locations. Given the inconsistency of LD for pairs of loci in each population, loci identified as linked in the global test were probably not truly linked. FST and RST estimates between Mottled Ducks and Mallards were low (0.047 and 0.080, respectively) but statistically significant. PCA analysis for Mottled Ducks and Mallards showed that individuals clearly formed 2 distinct clusters and there was obvious separation by species (Figure 2A).
After removing genetic hybrids identified by INSTRUCT, allelic richness for Mottled Ducks was 10.139 ± 1.056, while observed and expected heterozygosities were 0.5532 ± 0.0378 and 0.6703 ± 0.0345, respectively. Allelic richness for Mallards was 10.081 ± 0.895, while observed and expected heterozygosities were 0.5845 ± 0.0328 and 0.7083 ± 0.0345, respectively. Mottled Ducks showed linkage disequilibrium for 3% (20 out of 630) of Bonferroni-corrected pairwise comparisons among loci, whereas Mallards showed linkage disequilibrium for <1% (2 out of 630) of pairwise comparisons among loci. Locus pairs that showed LD (3%) in the global test for Mottled Ducks were in LD for only a few sampling locations (<3 locations; not Bonferroni corrected). One locus pair (Aful08 and Aful30) showed LD in 6 out of 12 locations. Given the inconsistency of LD for pairs of loci in each population, loci identified as linked in the global test were probably not truly linked. 

FST and RST estimates between Mottled Ducks and Mallards were low (0.052 and 0.086, respectively) but statistically significant. PCA analysis for Mottled Ducks and Mallards again showed individuals clearly forming 2 distinct clusters and obvious separation by species (Figure 2B).

**Genetic Admixture**

STRUCTURE HARVESTER indicated that the genotypic data best fit a 2-population model. The distribution of q-values showed 2 distinct clusters, where a q-value near 1 indicated that an individual was a Mottled Duck and a q-value near 0 indicated that an individual was a Mallard. In STRUCTURE, ~5% (22 out of 405) of ducks sampled in this study were deemed to be hybrids (mean q = 0.608 ± 0.056; Table 2). Ninety-five percent (303 out of 319) of putative Mottled Ducks were assigned to one genetic cluster (mean q = 0.984 ± 0.001), whereas 93% (71 out of 76) of putative Mallards were assigned to the other cluster (mean q = 0.016 ± 0.002). Ducks identified as Mallards in STRUCTURE were reanalyzed separately because they produced a more ‘scattered’ clustering in the PCA (Figure 2A), and to test whether we could determine wild and farm-raised Mallards. All birds but 5 were placed in 1 group. Two specimens (#337 and #338) were assigned to the ‘other’ population, but these were only genotyped at 19 and 21 loci out of 36, which may have caused the inconsistency. Another 3 specimens (#216, #362, and #398) had mixed assignments, but were mostly assigned as Mallards.

In INSTRUCT, ~8% (32 out of 405) of ducks sampled were deemed to be hybrids (mean q = 0.477 ± 0.053; Table 2). Ninety-five percent (303 out of 319) of putative Mottled Ducks were assigned to one genetic cluster (mean q = 0.983 ± 0.001; Table 2), whereas ~82% (62 out of 76) of putative Mallards were assigned to the other cluster (mean q = 0.025 ± 0.003). Overall, assignments by each program agreed 94% of the time (381 out of 405) for individual assignments. By

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<th>Genetic mixture assignment</th>
<th>Mallard</th>
<th>Mottled Duck</th>
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<tr>
<td>Mallard</td>
<td>76</td>
<td>71 (93%)</td>
<td>0</td>
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<tr>
<td>Mottled Duck</td>
<td>319</td>
<td>2 (1%)</td>
<td>303 (95%)</td>
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<td>Hybrid</td>
<td>10</td>
<td>3 (30%)</td>
<td>4 (40%)</td>
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<td>Mallard</td>
<td>76</td>
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species, there was a 96% (305 out of 317) agreement for putative Mottled Ducks between both programs, and 83% (63 out of 76) agreement for putative Mallards between both programs. Individuals identified as Mallards in the field were sometimes identified genetically as hybrids, but were never identified genetically as Mottled Ducks (Table 2). Individuals identified as Mottled Ducks or hybrids in the field were genetically identified as Mottled Ducks, Mallards, or hybrids (Table 2). In general, STRUCTURE assigned fewer putative Mallards as hybrids than INSTRUCT: 5 and 14 putative Mallards were assigned as hybrids by STRUCTURE and INSTRUCT, respectively (Table 2). Discrepancies between field identification and genetic assignments occurred rarely (3%; n = 4/135).

**DISCUSSION**

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 now regularly interact and hybridize with Mallards. Furthermore, released or escaped game-farm Mallards may account for many nonmigratory individuals that hybridize with resident species such as Mottled Ducks.

In the western Gulf Coast region, we estimated levels of Mallard–Mottled Duck hybridization at ~5–8%, a lower level than that observed in Florida (~9%; Williams et al. 2005). Low levels of hybridization may occur if hybrids of the heterogametic sex (females in ducks) are less fit and thus are absent, rare, or sterile (Haldane’s rule; Kirby et al. 2004). Kirby et al. (2004) observed Haldane’s rule in controlled, interspecific mating between captive populations of American Black Ducks and Mallards, and observed fewer F1 females on the breeding grounds in the subsequent year. Hybrid fitness has not been examined in western Gulf Coast Mottled Duck–Mallard crosses, but, since birds in the SACZ population seem to be admixed (Williams et al. 2005, Peters et al. 2016), any reduction in hybrid fitness is probably slight and unlikely to account for the low numbers of hybrids observed in the western Gulf Coast region.

**Mechanisms for Hybrid Production**

Low levels of hybridization between western Gulf Coast Mottled Ducks and Mallards may be best explained by limited opportunities for interaction as a consequence of each species’ breeding biology. Mottled Ducks are year-round residents of the western Gulf Coast, nesting primarily in coastal marsh and river delta habitats, but also in agricultural fields with lightly grazed or ungrazed vegetation (Durham and Afton 2003). In contrast, Mallards are migratory and breed throughout the U.S. (primarily in the prairie pothole region), except for sections of the southeastern states and coastal Louisiana and Texas (Baldassarre 2014). In addition to being historically absent from the Gulf Coast during the breeding season (Baldassarre 2014), Mallards have rarely been observed in coastal habitats during the western Gulf Coast Mottled Duck survey conducted annually in early April (L. Reynolds personal communication). Furthermore, pair formation in Mottled Ducks occurs in fall (as early as August, increasing through September, and with the majority paired by December; Baldassarre 2014); consequently, most Mottled Ducks (93–96%) may already be paired by the time that migratory Mallards arrive on the wintering grounds in late November through December (Baldassarre 2014). The lack of spatial and temporal overlap in Mallard and Mottled Duck breeding biology potentially limits opportunities for hybridization (Bielefeld et al. 2010).

Despite limited opportunities for interaction between Mottled Ducks and Mallards, hybridization does occur and could be explained in 4 ways: (1) mispairing during the fall and winter when pair formation normally occurs; (2) interspecific brood pooling; (3) interspecific forced copulations; and/or (4) rare migratory Mottled Ducks that produce hybrid young, which later breed on western Gulf Coast breeding grounds.

Mispairings, which happen for reasons including mistakes in mate recognition (Randler 2002) and/or a scarcity of conspecifics, may occur between native and introduced species when they have similar body sizes (Crespi 1989), plumage or coloration, and courtship displays. All of these conditions exist for Mottled Ducks and Mallards, including courtship displays that are nearly identical between the 2 species (Moorman and Gray 1994). Paulus (1988) observed Mottled Ducks and Mallards courting near each other in Louisiana; however, only 0.4% of Mottled Ducks paired with another species, and thus early pair formation in Mottled Ducks probably limited hybridization (Bielefeld et al. 2010). Individuals may also choose heterospecific mates when conspecifics are absent or already paired (Randler 2008). Because few Mallards are present during peak pair formation and they migrate north before or during the Mottled Duck breeding season, any remaining Mallards may have limited opportunities to mate conspecifically, and so choose heterospecifics rather than remaining unpaired and abandoning breeding altogether (Randler 2008).

Alternatively, interspecific brood pooling, a behavior more common in waterfowl than in any other avian order (Eadie et al 1988, Beauchamp 1998, Randler 2005), could result in hybridization if foster hatchlings sexually imprint on the foster female and later choose this species as a mate (ten Cate and Vos 1999, Randler 2005). Forced extrapair
copulations (FECs), a common secondary reproductive tactic in male waterfowl (McKinney et al. 1983, Davis 2002), may also account for hybridization between Mottled Ducks and Mallards. FECs by male mallards have been observed not just with female Mallards (Seymour 1990) but also with females in the Mallard complex (e.g., American Black Ducks in Nova Scotia; Seymour 1990). No direct information exists regarding FECs between Mallards and Mottled Ducks, but Coker et al. (2002) demonstrated that penis morphology (length, ridges, and knobs) predicted the frequency of forced copulations among waterfowl species. Using this approach, FECs are expected to occur infrequently in Mottled Ducks (Moorman and Gray 1994) and frequently in Mallards (McKinney and Evarts 1998). As Mallard penis morphology (Coker et al. 2002) is probably similar to that of closely related Mottled Ducks, and since FECs frequently occur in Mallards (Seymour 1990, Davis 2002, Randler 2005), they may also occur in Mottled Ducks, albeit perhaps less commonly. Overall, brood pooling appears to have a stronger impact on natural hybridization in waterfowl than FECs (Randler 2005), though data for either mechanism is lacking for western Gulf Coast Mottled Ducks.

Finally, hybridization may occur if Mottled Ducks follow and breed with Mallards on northern breeding grounds. If migratory Mottled Ducks produce hybrid young that return to wintering grounds in the western Gulf Coast region and remain there to breed, backcrossing could occur between Mottled Ducks and Mallards. Although a potentially rare occurrence, Mottled Ducks have been detected on northern breeding grounds (Selman et al. 2011).

When hybridization does occur in the western Gulf Coast region, it is unclear whether Mottled Ducks are hybridizing with migratory or nonmigratory feral or wild Mallards. Potential sources of feral Mallards include game breeders and preserves. In Louisiana, as of August 2015, there were only 5 game-breeder permits that reported having Mallards, and only 2 preserves that were permitted to keep Mallards (M. Collins personal communication). If Mallards escape from breeders or preserves, they could mate with wild Mottled Ducks and become migratory or nonmigratory birds. However, the number of feral Mallards in the western Gulf Coast region is unknown.

In this study, shared ancestry in ducks genetically identified to a species occurred as a result of the cutoff that we chose to identify species ($0.10 \geq q \geq 0.90$). This cutoff was chosen for consistency with Williams et al. (2005), and was needed because no ducks had 100% assignment to a single species. Because Mottled Ducks and Mallards are closely related, incomplete lineage sorting may account for shared ancestry. Lavretsky et al. (2014) estimated that Florida Mottled Ducks diverged from Mallards 390,000 yr ago, whereas western Gulf Coast Mottled Ducks diverged from Mallards only 245,000 yr ago. Our estimate of population differentiation between Mottled Ducks and Mallards in the western Gulf Coast region was significant but low ($F_{ST} = 0.047–0.052$), and was comparable with estimates published by Peters et al. (2016; $\Phi_{ST} = 0.034$) and Lavretsky et al. (2014; $\Phi_{ST} = 0.024$). Such congruence between microsatellite and SNP results is consistent with a recent study that showed that estimates of genetic variation obtained from microsatellites and SNPs were highly correlated, and that estimates of $F_{ST}$ may be similar despite large discrepancies in the number of each marker type (Elbers et al. 2017).

Although we showed with PCA analysis that the 2 species in our study were clearly separated, recent and rapid radiation together with ongoing gene flow between Mallards and other members of the Mallard complex probably contribute to weak differentiation between species (Lavretsky et al. 2014). Some shared ancestry may also be the result of hybridization that took place in the distant past (late stage backcrossing) between Mottled Ducks and Mallards. Lavretsky et al. (2016) used a simulated breeding experiment between Greater Scaup (Aythya marila) and Lesser Scaup (A. affinis) to create generational hybrids (F1–F10), and found that individuals from F1, F2, F3, and most F4 hybrid classes were distinguishable from ‘pure’ individuals. However, detection of hybrids in F3+ generations was not reliable and assignment values of $q = 0.01–0.04$ were consistent with hybrid ancestry including and beyond the F3 generation. This may also be true for Mottled Duck–Mallard hybrids, that is, F3+ hybrids that have backcrossed with a parental species may be indistinguishable from individuals of the parental species with no history of hybridization. Determining the generation of our hybrids is difficult because we cannot assume that we had any ‘pure’ parental individuals. However, based on the analysis conducted by Lavretsky et al. (2016), we considered individuals with ancestry coefficients of $0.10 \leq q \leq 0.90$ to be reliable F1 and F2 hybrids.

**Misidentifications and Assignment Discrepancies**

Two putative Mottled Ducks were genetically assigned as Mallards in this study; however, only one whole carcass was available for analysis of phenotypic hybrid characteristics using a recently published key (Bielefeld et al. 2016). This male specimen had phenotypic characters that identified it as a Mallard or hybrid (the key cannot distinguish between Mallards and hybrids; Bielefeld et al. 2016), suggesting that the bird was misidentified in the field. Mottled Ducks and Mallards can be difficult to distinguish because the females of both species resemble each other throughout the year. Additionally, male Mallards in drab plumage that are transitioning into
breeding plumage could have feather characteristics indicative of hybrids (e.g., green feathers on the head) and thus could be misidentified as Mottled Ducks or hybrids. In this study, genetic contradictions to key assignments were rare (3%; 4/135 were assigned incorrectly; Ford 2015) and were due to ducks lacking hybrid characteristics, such as green feathers on the head for males or white >=4 mm on the trailing edge of the speculum in females (Bielefeld et al. 2016). Misidentification also occurred when only a wing was available and the wing lacked hybrid characteristics, but was genetically identified as belonging to a hybrid.

Five putative Mallards were genetically assigned as hybrids by STRUCTURE and 14 putative Mallards were genetically assigned as hybrids by INSTRUCT, but 42% and 48% of loci did not amplify for 2 of the 5 individuals assigned as hybrids. During preliminary STRUCTURE analyses, we observed that using a small number of loci (e.g., n = 15–20) produced mixed ancestry for all individuals; therefore, these 2 individuals may have been genetically misclassified as hybrids simply because an insufficient number of loci was genotyped. The remaining 3 specimens had nearly complete genotypes (>94% of all loci amplified), which suggests that these hybrids may have been misidentified as Mallards in the field. Three putative Mallards (#381, #389, and #383; Supplemental Material Table S1) assigned as hybrids by STRUCTURE were also assigned as hybrids by INSTRUCT. Of the additional 9 putative Mallards assigned as hybrids by INSTRUCT, most individuals had almost complete genotypes (>94% of all loci amplified). An additional STRUCTURE analysis was performed for ducks assigned as Mallards by INSTRUCT because they produced a more ‘scattered’ PCA (Figure 2A), and they could have been either wild or farm-raised Mallards. However, this analysis was not sufficient to determine whether ducks identified as Mallards were wild or farm-raised, presumably because we did not have enough genetic information for farm-raised Mallards.

Interestingly, only 3 of the ducks identified as hybrids in the field (n = 10) were assigned as hybrids following genetic mixture analysis using STRUCTURE, while 4 were identified as hybrids using INSTRUCT (Table 2). In both STRUCTURE and INSTRUCT, the same 4 putative hybrids were genetically assigned as Mottled Ducks. In STRUCTURE, 3 putative hybrids were genetically assigned as Mallards; however, in INSTRUCT, only 2 of these 3 putative hybrids were genetically assigned as Mallards. Overall, this suggests that there may be confusion about the morphological and phenotypic characteristics that indicate hybrids in the western Gulf Coast region. Hybrids were distributed across the area of study without any obvious pattern with respect to region, habitat type, or state (Supplemental Material Table S1).

**Influence of Habitat**

It is unclear whether hybridization between Mottled Ducks and Mallards in the western Gulf Coast region will be a conservation issue in the future. Currently, habitat loss appears to be the leading factor limiting the survival and recruitment of western Gulf Coast Mottled Ducks (Wilson 2007): ~487,695 hectares of coastal marsh were lost in Louisiana between 1932 and 2010 (Couvillion et al. 2011), and 320,000 hectares have been lost in Texas since the 1950s (Moulton et al. 1997). However, ongoing habitat loss could indirectly lead to hybridization if Mottled Ducks respond by occupying wetlands found in urban and suburban areas, where nonmigratory Mallards may be more likely to occur (e.g., sightings reported in eBird of Mallards in the Gulf Coast region from April to August, 2005–2015, were primarily in cities; http://www.ebird.org). For example, Florida Mottled Ducks in the Upper St. Johns River Basin moved to wetlands associated with urban areas in response to reduced wetland availability in rural areas (Bielefeld and Cox 2006). Moreover, the majority of Mottled Ducks that moved to urban areas remained there, despite improved conditions in rural areas the following year (Bielefeld and Cox 2006).

In Florida, hybridization appears to be a serious threat to Mottled Ducks due to large numbers of nonmigratory Mallards and habitat selection by Florida Mottled Ducks. Mottled Ducks inhabit peninsular Florida south of Alachua County (Bielefeld et al. 2010) and are common in the wetlands of Lake Okeechobee and the Everglades Agricultural Area (Baldassarre 2014). However, unlike Gulf Coast Mottled Ducks, Florida Mottled Ducks (possibly more than half of the population) inhabit urban areas, where high concentrations of Mallards also congregate (Bielefeld et al. 2010). Additionally, Florida Mottled Ducks captured in urban and suburban areas showed a propensity for occupying artificial ponds and ditches (Bielefeld 2011), which may elevate hybridization levels because Mottled Ducks are more likely to encounter and hybridize with nonmigratory Mallards in these areas (FFWCC 2011). In contrast, Mottled Ducks in the western Gulf Coast region use fresh, intermediate, and brackish coastal marshes (Baldassarre 2014), and appear to avoid urban or rural areas.

Although anthropogenic changes that cause extensive hybridization between 2 distinct species may produce a hybrid swarm and the loss of distinct genetic lineages, limited long-term hybridization may result in increased genetic diversity. For instance, Peters et al. (2014) found nearly as much genetic diversity in western Gulf Coast Mottled Ducks as in North American Mallards, although Mallards had a much higher population census (11.6 million Mallards vs. ~159,000 Mottled Ducks; Zimpher et al. 2015 and USFWS 2015, respectively) and effective population size than Mottled Ducks (N_e = 2,400,000).
Mallards and 120,000 Mottled Ducks; Kraus et al. 2012, Peters et al. 2014). Our estimates of genetic diversity in each species mirrored the results of Peters et al. (2014), that is, levels of allelic richness and expected heterozygosity were both very similar between Mottled Ducks and Mallards. High genetic diversity in western Gulf Coast Mottled Ducks may have occurred due to some long-term gene flow between the 2 species (Peters et al. 2014).

Management Recommendations
Mottled Ducks and Mallards may have a long history of hybridization (Peters et al. 2014); therefore, some level of hybridization is probably normal and of little management concern. But what is a typical or acceptable level of hybridization between Mottled Ducks and Mallards in the western Gulf Coast region? As no baseline data exist prior to large-scale habitat loss and modification, setting an acceptable level of hybridization would be arbitrary. Monitoring changes in hybridization over time and responding to increases would probably be a more productive approach, and could be done by estimating metrics such as the proportion of hybrids in a sample and the mean proportion of ancestry in individuals (a measure of the ‘amount’ of Mottled Duck and/or Mallard in each individual).

Changes in the proportion of hybrids over time can be estimated by counting the number of hybrids in a sample at regular time intervals. Species and hybrids can be identified using genetic data where, for consistency with this study, hybrids are defined as those individuals having <90% ancestry from either species. Species and hybrids may also be identified via morphological traits. A recently developed phenotypic key effectively distinguishes Mottled Ducks from Mallards or hybrids (Bielefeld et al. 2016). However, the key is not designed to distinguish between Mallards and hybrids, although some characteristics have 3 states corresponding to Mottled Ducks, Mallards, and hybrids, respectively (e.g., Mottled Ducks have flat central tail feathers, whereas those of Mallards are curled, and those of hybrids are slightly raised; Bielefeld et al. 2016). Accurate estimates of the number of Mottled Ducks can be obtained using this key, but some error in the numbers of hybrids and Mallards would remain.

Our results and those of other studies (e.g., Wilson and Rohwer 1995) have shown that birds are sometimes misidentified in the field. In our study, birds were most often misidentified as hybrids when they were really Mottled Ducks or Mallards, although Mallards were sometimes misidentified as hybrids and Mottled Ducks were sometimes misidentified as hybrids or Mallards (Table 2). Collecting data on the proportion of hybrids via morphological identification will be most feasible at hunter check stations because daylight and the Bielefeld et al. (2016) key will help to ensure accurate identification. Collecting these data during banding operations will be less feasible as time is limited and low light levels may increase misidentification.

Changes over time in mean ancestry proportions could also be used to monitor hybridization. In this study, the mean q-value obtained for Mottled Ducks was 0.984 using STRUCTURE and 0.983 using INSTRUCT; that is, individuals genetically designated as Mottled Ducks (≥90% Mottled Duck ancestry) had, on average, 98% Mottled Duck ancestry. If the mean q-value were to show a trend toward 90% over time, then hybridization could be said to be increasing.

We suggest that levels of hybridization be reassessed genetically every 5 yr and monitored annually at hunter check stations via morphological identification. This timescale would allow increases in hybridization to be observed quickly without incurring great cost. If future genetic estimates are intended to be compared with those in this study, they should be calculated with the same panel of microsatellite loci, as changes in the number or identity of loci can dramatically change genetic estimates.

Protecting coastal marsh habitat may also help to prevent increases in hybridization if it prevents Mottled Ducks from occupying urban areas where Mallards occur. Because Louisiana has lost a considerable amount of coastal wetland area (Couvillion et al. 2011), the Louisiana Coastal Protection and Restoration Authority has developed a statewide master plan to slow coastal erosion and to achieve no net annual loss of wetlands by 2032–2041 (Coastal Protection and Restoration Authority of Louisiana 2012). Restoration measures outlined in the master plan (e.g., coastal erosion barriers, marsh creation projects, and hydrologic restoration) could improve the long-term outlook for persistence of Mottled Ducks in coastal marsh habitats of Louisiana. However, many of the strategies proposed in the master plan have yet to be evaluated for their value for restoring wildlife habitats, and therefore additional research is needed to investigate their value to Mottled Ducks.

Our genetic analysis of western Gulf Coast Mottled Ducks and North American Mallards showed that current levels of hybridization appear to be low overall. These hybridization levels are probably of limited conservation concern, given that (1) Mottled Ducks and Mallards may have a long history of limited hybridization and therefore some hybridization may be expected; (2) western Gulf Coast Mottled Ducks avoid urban and suburban habitats where Mallards may congregate; and (3) Mallards are relatively rare in western Gulf Coast Mottled Duck habitat during pair formation and the breeding season. However, periodic monitoring of hybridization will be critical to ensure that hybridization does not increase should ongoing habitat loss and modification drive Mottled Ducks into urban wetland habitats, where they may be
more likely to encounter and hybridize with nonmigratory Mallards.

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