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Molecular population genetics, phylogeography, and conservation biology of the mottled duck (*Anas fulvigula*)

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Abstract

The mottled duck (*Anas fulvigula*) is a year-round endemic resident of the Gulf Coast and one of two non-migratory dabbling ducks that inhabit North America. To investigate population genetic structure of allopatric mottled duck populations, we collected 5' control region sequences (bp 78–774) from the mitochondria of 219 mottled ducks sampled at 11 widely spaced geographic localities in Texas, Louisiana, and Florida and compared them to each other and to homologous sequences from 4 Mexican ducks (*A. diazi*), 13 American black ducks (*A. rubripes*), and 10 mallards (*A. platyrhynchos*). We identified 57 unique haplotypes composed of 665 or 666 nucleotides in the 246 control region sequences. Of the 665 homologous positions, 8.3% (n = 55) vary among haplotypes, and 98.2% (n = 54) of these occur within the first 351 nucleotides from the 5' end of the outgroup sequence. Neighbor-joining analysis shows a large distal clade (52.5% of mottled ducks sampled in our study) composed of two reciprocally monophyletic clades of mottled duck haplotypes, one of which is endemic to Texas and Louisiana and the other endemic to Florida. No mottled ducks sampled in Florida occur in the clade composed of mottled ducks from Texas and Louisiana or vice versa, suggesting that (1) an enduring geographic split has existed for many years between east and west, and (2) gene flow currently is non-existent (or at least undetectable) across the central Gulf Coast. The remaining 47.5% of mottled ducks sampled in our study branch basally from this derived clade, show substantially less hierarchical structure, and fall into various lineage groups of mixed species composition with no geographic or species-specific pattern. Pairwise F_{ST} values corroborate the pattern of strong differentiation observed between Texas/Louisiana and Florida. Our findings are consistent with a pattern of partial lineage sorting from a polymorphic ancestral gene pool reshuffled by hybridizing mallards. Control region data and patterns of divergence in mallard-like species worldwide, furthermore, suggest that mottled ducks are close relatives of Mexican ducks, and in turn nested within black ducks. Genetic similarities to nominate mallards are less likely to be the product of common ancestry, but the result of past hybridization with a dichromatic mallard ancestor that invaded North America from Asia many generations ago. Our findings have several important consequences for the conservation biology of mottled ducks across the Gulf Coast and our understanding of the phylogeography of mallard-like species worldwide.

Introduction

Geographic patterns of genetic variation within and among animal populations have long been of interest to evolutionary geneticists and conservation biologists (Avise 1994, 2000). Geographic barriers to gene flow and their persistence through time, in particular, are at the root of the biological species concept (e.g., Mayr 1963), and evidence of reduced gene flow within a species underlies our concepts of populations, subspecies, super-species, and other units believed to be evolving along divergent evolutionary trajectories

(Moritz 1994; Barrowclough and Flesness 1996). For species with limited geographic ranges and small or declining populations, historical patterns of demography and hierarchical genetic structure are important elements not only in determining the population structure, but also to be considered in the development of an effective and sustainable management plan. In this paper, we investigate questions related to gene flow, genetic structure, and phylogeography of a relatively small population of recreationally harvested dabbling ducks, the mottled duck (*Anas fulvigula*).

The mottled duck is a year-round endemic resident of the Gulf Coast and one of two non-migratory dabbling ducks that inhabit North America (Bellrose 1976) the other is the similarly plumaged Mexican duck (*A. diazi*). Two populations of mottled ducks occur on the Gulf Coast; one is a resident of peninsular Florida and the other is a resident of the Gulf Coast from Alabama westward to Veracruz, Mexico (Moorman and Gray 1994; Figure 1). The last edition of the American Ornithologists' Union's (1957) *Checklist of North American Birds* to include sub-species recognized two for mottled ducks, *A. f. fulvigula* in Florida and *A. f. maculosa* in the western areas of the range. Information published on the morphology and plumage characteristics of the two populations suggests that slight differences may exist between the two populations (Bellrose 1976; Stutzenbaker 1988; Moorman 1991; Gray 1993), but no one has examined geographic variation in detail (Moorman and Gray 1994). Banded birds from both populations have been recovered north of their normal range on rare occasions; however, there are no records of exchange of individuals between the two populations (Moorman and Gray 1994).

The mottled duck population in Florida appears to be relatively stable, hovering near a population of 56,000 in the fall (Johnson et al. 1984; Brust 1993). Even so, there is growing concern about the status of the mottled ducks in Florida. Pressure on mottled duck habitat from urbanization and agriculture is intense, and hybridization with feral mallards (*A. platyrhynchos*) appears to be a growing problem. In fact, Moorman and Gray (1994) suggest that the potential for hybridization in Florida is so great that the mottled duck's status as a species is threatened. In contrast, Texas and Louisiana populations appear to be stable (McKenzie et al. 1988), and preliminary data suggest that the population inhabiting those states probably is much larger than Stutzenbaker's (1988) maximum estimate of 169,300 based on mid-winter inventory data (B. Wilson, Gulf Coast Joint Venture unpubl. data).

Uncertainty about sub-specific designations for mottled duck populations has the potential to influence the management of this species, including harvest, across populations. Should one population decline to the point where restrictive harvest measures or season closures are warranted, it is conceivable that U.S. Fish and Wildlife Service policy would require solid evidence of population subdivision before allowing central Gulf Coast populations to continue to be managed separately from those inhabiting Florida. Despite such concerns, no information is available to indicate whether the two mottled duck populations are distinct.

To answer this and other questions related to the possible existence of localized patterns of gene flow within each area of geographic sub-division, we collected mottled ducks at widely spaced geographic localities in Texas, Louisiana, and Florida and sequenced the 5' half of the control region of the maternally-inherited mitochondria genome (chicken mtDNA positions 78–774; Desjardins and Morais 1990). Our sequences span most of domains I (the 5' variable region) and II (the central domain) of the avian control region (Quinn and Wilson 1993; Marshall and Baker 1997). We also included Mexican ducks, American black ducks (*A. rubripes*), and mallards in our analysis, and in doing so employed a molecular phylogenetic approach to understanding the genetic relationships of the four mallard-like species that inhabit North America. We discuss the mottled duck's apparent relationships to other mallard-like species, identify alternative explanations for observed patterns of lineage radiation and introgression, and make recommendations relevant to the conservation and management of mottled ducks inhabiting the central Gulf Coast and Florida.

Materials and methods

Collection, amplification, and sequencing

We obtained feathers from wings of 202 mottled ducks collected by hunters during the 1998–1999 hunting season in 11 widely spaced geographic localities in Texas, Louisiana, and Florida (Figure 1, Table 1). Each mottled duck wing was scored for the presence of possible mottled duck × mallard hybrid characters including additional white feathers in the trailing edge

Figure 1. Geographic ranges of the mottled duck and the Mexican duck. Mottled ducks also occur in small numbers in Mississippi and Alabama, and have been recorded outside their normal range in north Texas, Oklahoma, Colorado, Kansas, Iowa, and Wisconsin. A population derived from released mottled ducks originally obtained from Texas, Louisiana, and Florida also exists in South Carolina and Georgia (Stutzenbaker 1988). Sampling localities for mottled ducks and Mexican ducks are numbered west to east and include: 1 = San Luis Potosí, México; 2 = Laguna Atascosa NWR, TX; 3 = Mad Island WMA, TX; 4 = Peach Point WMA, TX; 5 = Anahuac NWR, TX; 6 = J. D. Murphree WMA, TX; 7 = Sabine NWR, LA; 8 = Lacassine NWR, LA; 9 = Atchafalaya Delta WMA, LA; 10 = Lake Josephine, FL; 11 = Lake Istokpoga, FL; and 12 = T. M. Goodwin WMA, FL.

of the speculum and the presence of white feathers in the leading edge of the speculum (e.g., Stutzenbaker 1988). Tail feathers were obtained from another 17 mottled ducks banded at Laguna Atascosa National Wildlife Refuge (NWR), Texas, during August 1999. These mottled ducks were undergoing wing molt and were not scored for hybrid characters. Feathers or genomic DNA extracts were obtained from an additional 4 Mexican ducks collected in San Luis Potosí, México, between 1950 and 1952, 13 black ducks, and 10 mallards from various other sources for a total of 246 individuals in all (Table 1). DNA subsequently was isolated from the base of one or two feather quills from each individual using a DNeasy Tissue Kit (QIAGEN Inc., Valencia, California). Thirty μl of 100 mg/ml DTT (dithiothreitol) were added to the digestion buffer to dissolve feather quills. Next, we used PCR (e.g., Gyllensten 1989) to amplify the 5' half of the mitochondrial DNA control region (bp 78–774 in the chicken mitochondrial genome; Desjardins and Morais 1990). Control region primers included Sorenson and Fleischer's (1996) L78 and Sorenson et al.'s (1999) H774. We used L78 and H493 (Sorenson and Fleischer 1996) for the four Mexican ducks, because amplification of the L78–H774 fragment was not successful. PCR reactions were carried out in a GeneAmp PCR System 2400 oil-free thermalcycler (Perkin Elmer Applied Biosystems, Norwalk, Connecticut) using a 50 μl reaction containing 2 μl template DNA, 2.5 μl of each primer (10 μM), 5 μl of 10 μM dNTPs, 5 μl of 25 mM $MgCl_2$, 5 μl of 10X PCR buffer, and 0.25 U Taq Polymerase (Perkin Elmer Applied Biosystems, Norwalk, Connecticut). Thermal cycling was as follows: 7 min pre-heat at 94 °C, followed by 45 cycles of 20 s at 94 °C, 20 s at 52 °C, 1 min at 72 °C, and a final extension of 7 min at 72 °C.

The entire contents of each PCR product were electrophoresed in 1% agarose at 100–120 volts for 1 hour, stained with 10 μg/μl ethidium bromide,

Table 1. Species, geographic localities, number of individuals sampled, and sources of genetic material included in this study

Species	Geographic locality	No. individuals	Source of genetic material
Mottled duck (*Anas fulvigula*)	Laguna Atascosa National Wildlife Refuge, Cameron County, Texas, USA	17	Feathers, USFWS leg-banded birds, August 1999
	Mad Island Wildlife Management Area, Matagorda County, Texas, USA	27	Feathers, Hunter check stations, 24 Oct. to 21 Nov. 1998
	Peach Point Wildlife Management Area, Brazoria County, Texas, USA	28	Feathers, Hunter check stations, 17 Oct. to 12 Dec. 1998
	Anahuac National Wildlife Refuge, Chambers County, Texas, USA	30	Feathers, Hunter check stations, 24 Oct. 1998 to 9 Jan. 1999
	J. D. Murphree Wildlife Management Area, Jefferson County, Texas, USA	26	Feathers, Hunter check stations, 24 Oct. to 7 Nov. 1998
	Sabine National Wildlife Refuge, Cameron Parish, Louisiana, USA	24	Feathers, Hunter check stations, 8 Nov. to 15 Nov. 1998
	Lacassine National Wildlife Refuge, Cameron Parish, Louisiana, USA	23	Feathers, Hunter check stations, 12 Nov. 1998 to 7 Jan. 1999
	Atchafalaya Delta Wildlife Management Area, St. Mary Parish, Louisiana, USA	11	Feathers, Hunter check stations, 7 Nov. to 21 Nov. 1998
	Lake Josephine, Highlands County, Florida, USA	1	Feathers, Hunter check stations, 14 Jan. 1999
	Lake Istokpoga, Highlands County, Florida, USA	2	Feathers, Hunter check stations, 10 Dec. 1998 to 7 Jan. 1999
	T. M. Goodwin Waterfowl Management Area, Brevard County, Florida, USA	30	Feathers, Hunter check stations, 21 Nov. 1998 to 19 Jan. 1999
Black duck (*Anas rubripes*)	Nova Scotia, Canada[a]	1	Genomic DNA extract, Judith Rhymer, University of Maine
	Petit Manan National Wildlife Refuge, Washington County, Maine, USA	2	Genomic DNA extract, Judith Rhymer, University of Maine
	Eastern Shore, Maryland, USA[b]	1	Genomic DNA extract, Judith Rhymer, University of Maine
	Atlantic Flyway, USA[c]	9	Feathers, Atlantic Flyway Wing Bee, Paul Padding, Office of Migratory Bird Management, USFWS
Mexican duck (*Anas diazi*)	San Luis Potosí, San Luis Potosí, México	4	Feathers, LSUMNS[d] 14897, 18557, 18558, 21121
Mallard (*Anas playrhynchos*)	Central Flyway, USA[c]	6	Feathers, Central Flyway Wing Bee, Brian Sullivan, Texas Parks and Wildlife
	Suisun Marsh, Solano County, California, USA	3	Genomic DNA extract, Judith Rhymer, University of Maine
	Deale, Anne Arundel County, Maryland, USA	1	Genomic DNA extract, Judith Rhymer, University of Maine

[a]Captive breeding flock at Patuxent Wildlife Research Center, Laurel, Maryland, 1989.
[b]Captive breeding flock at National Zoological Park, Front Royal, Virginia, 1989.
[c]Specific localities within the flyways are unknown.
[d]Louisiana State University Museum of Natural Science.

excised, and gel-purified using a QIAquick Gel Extraction Kit (QIAGEN Inc., Valencia, California). Light and heavy strand PCR products subsequently were cycle-sequenced in 10 μl reactions using four-fold diluted BigDye Terminator Cycle Sequencing Kits and a GeneAmp PCR System 2400 (Perkin Elmer Applied Biosystems, Norwalk, Connecticut), followed by electrophoresis on an ABI 377 automated DNA-sequencer (Perkin Elmer Applied Biosystems, Norwalk, Connecticut). Sequences from opposite strands were reconciled using Sequencher 3.1 (Gene Codes Corporation, Ann Arbor, Michigan), verified for accuracy, and aligned by eye. Unique sequences are archived in GenBank (National Center for Biotechnology Information, Bethesda, Maryland) under accession numbers AF382404–382649.

Mottled duck phylogenetics and population genetics

Number of haplotypes and percent of variable nucleotide sites were measured within each mottled duck sampling locality. Haplotype diversity (h), nucleotide diversity (π) (Nei 1987, solutions to equations 8.5 and 10.5), and Tajima's (1989) D test for departure from neutrality were calculated for each locality using Arlequin ver2.0b2 (Schneider et al. 1999). For these three calculations genetic distances were corrected for multiple hits by the method of Kimura (1980) assuming a pre-determined gamma shape parameter (α) = 0.88 (alternative models such as HKY-85 are not available in Arlequin ver2.0b2; see below). We next used likelihood ratio tests implemented in the software program MODELTEST (Posada and Crandall 1998) to determine the appropriate, minimum-parameter, maximum-likelihood model for the sequence data optimized on a neighbor-joining tree constructed in PAUP* 4.0b1 (Swofford 1998). The most appropriate model was the Hasegawa et al. (1985) model, with adjustments for invariant sites and the gamma shape parameter (α). A second neighbor-joining tree using these starting parameters then was constructed with PAUP* 4.0b1 (Swofford 1998). Log-likelihoods for the resultant neighbor-joining tree subsequently were calculated using these parameters, empirical base frequencies, and an estimated ti:tv bias of 48.72:1. A molecular clock was not enforced. Ti:tv and α were reconfirmed iteratively by estimating one parameter and assuming it to estimate the other until both parameters stabilized. All trees were rooted on four divergent mallard haplotypes identified in five indi-

viduals among a total of ten mallards; the remaining five mallard haplotypes identified in five individuals clustered within the ingroup; see Avise et al. (1990), Cooper et al. (1996), Johnson and Sorenson (1999), and Rhymer (2001) for further reference to two divergent classes of mallard haplotypes.

We next performed analyses of molecular variance (AMOVA) (Excoffier et al. 1992) as implemented in Arlequin ver2.0b2 (Schneider et al. 1999) to determine if hierarchical structure existed among distant geographic locations. We also calculated population pairwise F_{ST} test statistics and corresponding p-values based on the AMOVA model. First, we performed a set of analyses for the mottled duck haplotypes pooled at different hierarchical, geographic levels (i.e., east vs. west, state vs. state, and localities within each state), and then we repeated the analyses for monophyletic groups shown in the neighbor-joining tree (Figures 2, 3). Genetic distances were corrected for multiple hits by the method of Kimura (1980) assuming a pre-determined gamma shape parameter (α) = 0.88. Pairwise mismatch distributions (Slatkin and Hudson 1991; Rogers and Harpending 1992; Harpending et al. 1993) and Roger's (1995) model of sudden population expansion also were calculated using Arlequin ver2.0b2 (Schneider et al. 1999). No corrections for multiple hits were included in pairwise mismatch calculations and sudden expansion models.

Reanalysis of mallard phylogenetic relationships

To better understand the relationship of mottled ducks to New World mallards and the mallard group as a whole, we reanalyzed Johnson's and Sorenson's (1999) ND2 and cytochrome *b* sequence data (2166 bp) for twelve of the world's fourteen extant or recently extinct mallard species and four green-winged teals. Because the original data set yielded three equally parsimonious trees when transversions were weighted 5:1 over transitions (see also Johnson and Sorenson 1999), we reanalyzed the data using maximum likelihood implemented in PAUP* 4.0b1 (Swofford 1998). Maximum likelihood analyses employed heuristic searches with tree bisection and reconnection branch swapping, repeated 100 times, initiating each search with a random addition sequence to ensure unbiased sampling of tree space. Empirical base frequencies, a ti:tv bias of 16.15:1, proportion of invariable sites (p-inv) = 0.79, and equal rates for all sites were defined a priori as parameters of a nucleotide substitution model corrected for multiple hits by the method of Hasegawa et al. (1985). Starting parameters were obtained from the best of three equally parsimonious trees, as determined by likelihood evaluations calculated in PAUP* 4.0b1 (Swofford 1998). Ti:tv and p-inv subsequently were reconfirmed iteratively by estimating one parameter and assuming it to estimate the others until all parameters stabilized. All trees were rooted on four green-winged teals (see Johnson and Sorenson 1999). Bootstraps were used to assess support for internal nodes for the maximum likelihood analysis (Felsenstein 1985; Hillis and Bull 1993).

Results

We identified 57 unique haplotypes composed of 665 or 666 nucleotides in the 246 control region sequences, or approximately one for every four individuals sampled; these include four divergent haplotypes identified in five mallard sequences used as the outgroup. A nucleotide deletion at position 211, relative to the 5' end of the mallard outgroup haplotype, was identified in each of the 241 mottled duck, Mexican duck, black duck, and mallard haplotypes observed in the ingroup. Of the 665 homologous positions, 55 (8.3%) varied among haplotypes. Transitions occurred at 53 (96.4%) of these positions, and transversions occurred at 2 (3.6%) positions. Fifty-three of 54 variable positions occurred within the first 351 nucleotides relative to the 5' end of the outgroup haplotype; the other variable position occurred at position 521. No ambiguous or co-amplified nucleotides were observed, and other evidence not included here suggests the haplotypes are of mitochondrial origin.

Within any given sampling locality, we identified four to sixteen unique haplotypes (Table 2). Proportions of variable sites ranged from 1.35% to 3.16% (Table 2). Haplotype diversity (h) and nucleotide diversity (π) were greatest at Peach Point Wildlife Management Area (WMA), Texas, and least at Atchafalaya Delta WMA, Louisiana. Variance estimates around nucleotide diversity were relatively wide, suggesting that more base pairs and individuals may be required to obtain precise estimates of nucleotide diversity. None of the sampling localities showed a significant departure from neutrality as indicated by Tajima's (1989) D (all Ps > 0.17; Table 2).

Haplotype relationships for all sampled individuals and their corresponding geographic localities

Table 2. Estimates of within population variability of 5' control-region sequences for mottled ducks by locality including number of individuals sampled, total number of haplotypes, percent of nucleotide positions that vary, haplotype diversity (h), nucleotide diversity (π), and Tajima's (1989) test for departure from neutrality

Sample locality	No. individuals	No. haplotypes	% variable sites	Haplotype diversity (h) $\pm$ $V(h)$[a]	Nucleotide diversity (π) $\pm$ $V(\pi)$[b]	Tajima's D[c]
Laguna Atascosa NWR, TX	17	9	2.11	0.8309 ± 0.0846	0.006385 ± 0.003727	0.01906
Mad Island WMA, TX	27	10	2.71	0.9060 ± 0.0267	0.006865 ± 0.003877	−0.13985
Peach Point WMA, TX	28	16	3.16	0.9471 ± 0.0235	0.007356 ± 0.004114	−0.39297
Anahuac NWR, TX	30	13	2.86	0.8897 ± 0.0336	0.006384 ± 0.003624	−0.45054
J. D. Murphree WMA, TX	26	9	2.71	0.8123 ± 0.0528	0.006512 ± 0.003707	−0.35398
Sabine NWR, LA	24	11	2.56	0.8877 ± 0.0450	0.007102 ± 0.004014	0.06145
Lacassine NWR, LA	23	9	2.26	0.7628 ± 0.0864	0.006281 ± 0.003612	0.02697
Atchafalaya Delta WMA, LA	11	4	1.35	0.6727 ± 0.1232	0.003492 ± 0.002330	−1.06930
T. M. Goodwin WMA, FL[d]	33	12	2.41	0.8542 ± 0.0455	0.006192 ± 0.003518	0.07436

[a]Nei (1987) equation 8.5.
[b]Nei (1987) equation 10.5.
[c]Tajima (1989; all Ps > 0.17).
[d]Also includes three individuals from Lake Josephine (n = 1) and Lake Istokpoga (n = 2), Highlands County, Florida.

are depicted in Figures 2 and 3 as determined by a neighbor-joining analysis corrected for multiple hits by the method of Hasegawa et al. (1985). Maximum likelihood parameters for this tree are: lnL = −1499.67, ti:tv = 48.72, p-inv = 0.81, and α = 0.88. Within the crown of the tree (Figure 2) is a large terminal clade composed of two reciprocally monophyletic groups of haplotypes, one of which is composed of 91 (48.9%) mottled ducks sampled in Texas and Louisiana and the other composed of 24 (72.7%) mottled ducks sampled in Florida. No mottled ducks sampled in Florida occur in the clade composed of mottled ducks from Texas and Louisiana, and no mottled ducks sampled in Texas or Louisiana occur in the clade composed of mottled ducks from Florida. Two mallards sampled in the Central Flyway occur in the clade composed of mottled ducks from Texas and Louisiana. Both lineages show substantial hierarchical structure within their respective clades, and the inter-node leading to the Florida haplotypes is the longest branch in the ingroup. The remaining 104 (47.5%) mottled ducks sampled in our study branch basally, show substantially less hierarchical structure (i.e., many are poorly sorted), and fall into various lineage groups of mixed species composition. No clear geographic or species-specific patterns are evident. The Mexican duck haplotype identified in four individuals from San Luis Potosí, México, is similar to mottled duck haplotypes sampled from Texas, Louisiana, and Florida and identical to a black duck haplotype collected from one individual in the Atlantic Flyway. The four outgroup haplotypes identified in five mallards differ from the closest members of the ingroup by ten to thirteen nucleotide substitutions and a thymine insertion at position 211, and correspond to Avise et al.'s (1990) class A mallard haplotype and Johnson's and Sorenson's (1999) type 1 mallard haplotype. Additional white feathers in the leading and trailing edges of the speculum (a putative mottled duck × mallard hybrid character; Stutzenbaker 1988) are not associated with any particular haplotype or group of haplotypes. However, 27.3% (9 of 33) of mottled ducks collected in Florida exhibited these characters, as did 17.9% (5 of 28) of mottled ducks collected at Peach Point WMA, Texas. Elsewhere in Texas and Louisiana, only 2.8% (4 of 141) of mottled ducks showed the hybrid characters.

Pairwise F_{ST} values corrected for multiple hits by the method of Kimura (1980) showed no significant patterns of differentiation between localities in the western part of the mottled duck range, as indicated by comparisons including all localities in Texas and the two Louisiana localities closest to Texas; F_{ST} = −0.0448 to 0.0242, all Ps > 0.15 (Table 3). In contrast, F_{ST} values calculated for Florida and each other locality indicated strong differentiation. Values ranged from 0.3841 to 0.5542 and were significant for all comparisons (all Ps < 0.0001) as determined by AMOVA (Excoffier et al. 1992). F_{ST} values for pairwise comparisons including Atchafalaya Delta, Louisiana, ranged from 0.1183 to 0.2564 and also were significant (all Ps ≤ 0.0254). AMOVA for

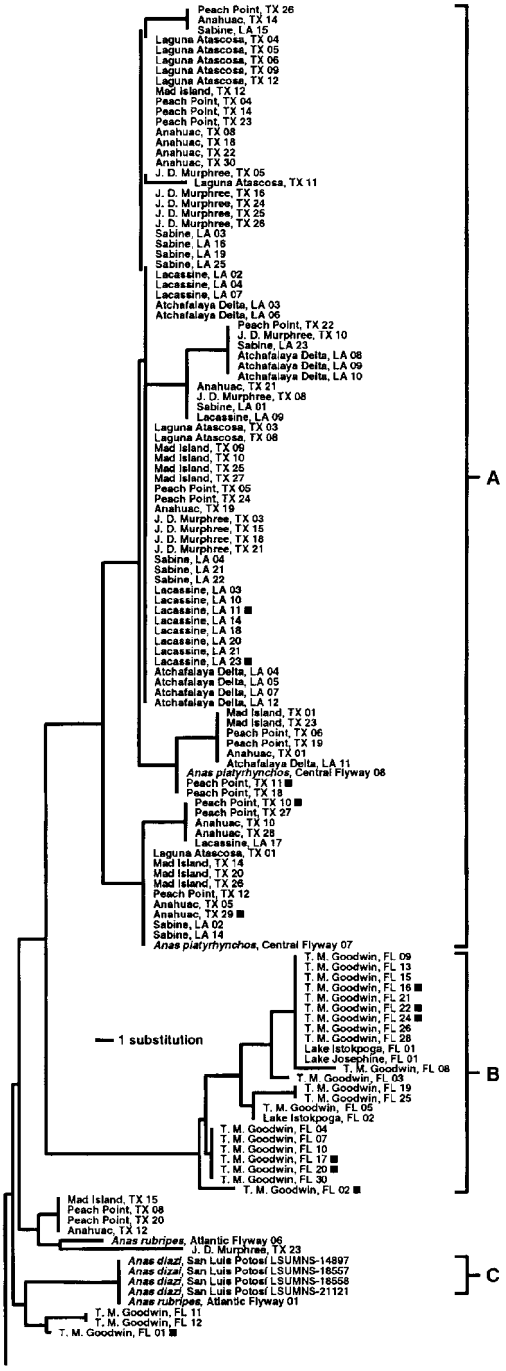

Figure 2. Upper half of neighbor-joining tree depicting the phylogenetic relationships of 246 mottled ducks, Mexican ducks, American black ducks, and mallards (lnL = −1499.67; ti:tv = 48.72; p-inv = 0.81; α = 0.88; see also Figure 3). (a) Texas/Louisiana mottled duck haplotypes, (b) Florida mottled duck haplotypes and (c) Mexican duck haplotype. Squares indicate individuals scored as possible mottled duck × mallard hybrids based on the presence of a white stripe on the leading edge of the speculum (mottled ducks at Laguna Atascosa NWR, Texas, were not checked for hybrid characters).

two groups (Florida vs. Texas/Louisiana) and nine populations indicated that 59.3% of overall variation occurred within populations, 39.6% among populations, and 1.1% between groups. The F_{ST} value contrasting the clades endemic to Florida and Texas/Louisiana (Figure 2) was 0.8317 ($P < 0.0001$). AMOVA for these two clades indicated that 83.2% of variation occurred between populations and 16.8% within populations.

Nucleotide mismatch distributions for mottled ducks, Mexican ducks, black ducks, and mallards of the ingroup are uni-modal and do not reject Rogers' (1995) model of sudden population expansion following a recent bottleneck ($P > 0.21$; Figure 4a). Nucleotide mismatches for the large clade of haplotypes endemic to Texas, Louisiana, and Florida show a multi-modal pattern and also do not reject Rogers' (1995) sudden expansion model ($P > 0.60$; Figure 4b). However, nucleotide mismatches for haplotypes endemic to Florida alone marginally reject the null hypothesis of recent population expansion ($P = 0.0670$; Texas and Louisiana $P > 0.33$).

Discussion

Mottled duck haplotype relationships

Mottled ducks sampled in our study show two distinct classes of haplotypes. The first class of haplotypes is located in the distal part of our tree, corresponds almost exclusively to mottled ducks, and is geographically structured (Figures 2, 3). All other mottled duck haplotypes sampled in our study branch basal to this derived clade, show substantially less phylogenetic structure, and fall into lineage groups of mixed species and population composition with no species-specific or geographic pattern. These two patterns are characteristic of a combination of modern and ancient population genetic processes, including (1) restricted gene flow across extant geographic barriers, (2) incomplete lineage sorting from a polymorphic ancestral gene pool, and (3) introgressive hybridization across species boundaries. The latter two processes are often indistinguishable, and we believe that some combination of all three have played important roles in the evolution of mottled duck populations.

Identification of a distal clade composed of two reciprocally monophyletic sister groups of mottled duck haplotypes (48.9% of the Texas and Louisiana samples and 72.7% of the Florida samples) suggests

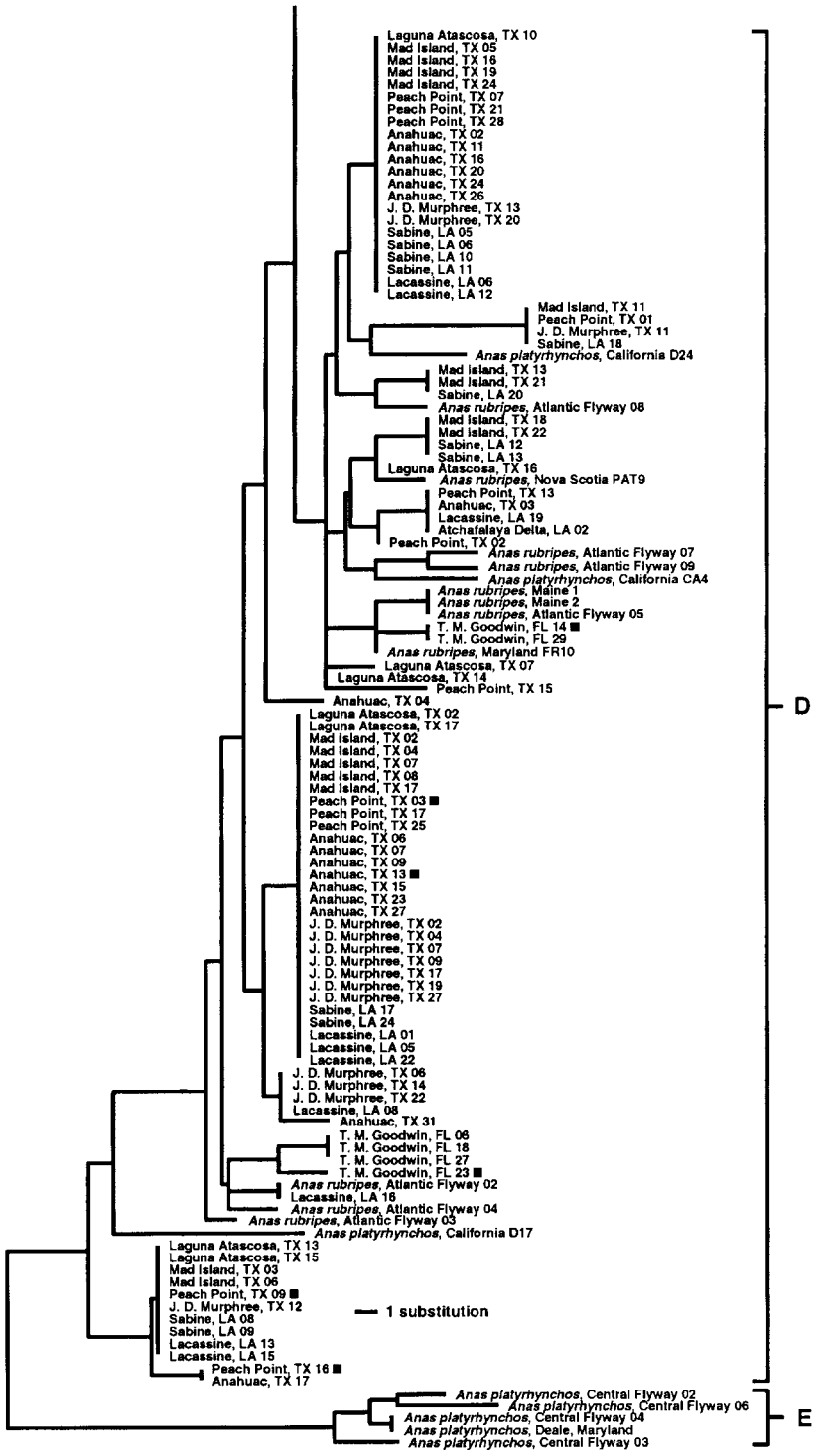

Figure 3. Lower half of neighbor-joining tree depicting the phylogenetic relationships of 246 mottled ducks, Mexican ducks, American black ducks, and mallards (lnL = −1499.67; ti:tv = 48.72; p-inv = 0.81; α = 0.88; see Figure 2). (d) Mixed species haplotypes and (e) mallard outgroup haplotypes. Squares indicate individuals scored as possible mottled duck × mallard hybrids based on the presence of a white stripe on the leading edge of the speculum (mottled ducks at Laguna Atascosa NWR, Texas, were not checked for hybrid characters).

Table 3. Pairwise F_{ST} values corrected for multiple hits by the method of Kimura (1980) (upper matrix) and corresponding F_{ST} *p*-values (lower matrix) for AMOVA (Excoffier et al. 1992) including two groups (east vs. west) and nine populations

Sample locality	Sample locality 1	2	3	4	5	6	7	8	9
1. Laguna Atascosa NWR, TX	–	0.0010	−0.0258	0.0042	−0.0094	−0.0376	−0.0448	0.1416	0.4036
2. Mad Island WMA, TX	0.3662	–	−0.0064	−0.0182	−0.0031	−0.0099	0.0242	0.2541	0.3841
3. Peach Point WMA, TX	0.8359	0.5205	–	−0.0063	−0.0038	−0.0222	−0.0156	0.1361	0.3956
4. Anahuac NWR, TX	0.3565	0.8174	0.5215	–	−0.0156	0.0039	0.0130	0.2565	0.3993
5. J. D. Murphree WMA, TX	0.4512	0.4102	0.4395	0.6865	–	−0.0008	−0.0084	0.2091	0.4177
6. Sabine NWR, LA	0.9365	0.5410	0.8809	0.3018	0.3779	–	−0.0215	0.1357	0.3908
7. Lacassine NWR, LA	0.9893	0.1504	0.6621	0.2275	0.4717	0.7275	–	0.1183	0.4235
8. Atchafalaya Delta WMA, LA	0.0234	0.0020	0.0059	0.0029	0.0078	0.0137	0.0254	–	0.5542
9. T. M. Goodwin WMA, FL[a]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	–

[a]Also includes three individuals from Lake Josephine (n = 1) and Lake Istokpoga (n = 2), Highlands County, Florida.

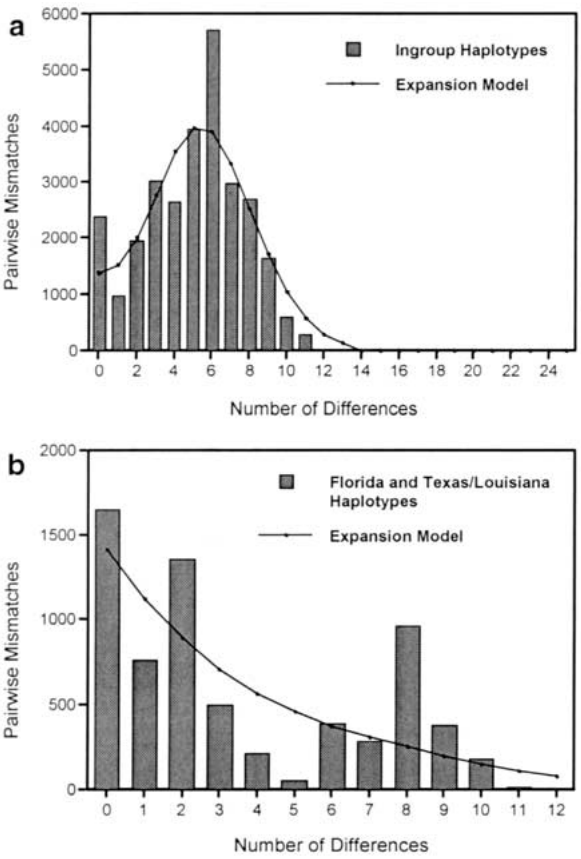

Figure 4. Nucleotide mismatch distributions for (a) all ingroup individuals and (b) individuals possessing mottled duck haplotypes endemic to Florida, Texas, and Louisiana; see Figures 2 and 3 for the composition of each clade. Solid lines indicate the patterns expected by Rogers' (1995) model of sudden expansion following a bottleneck.

that gene flow currently is non-existent (or at least undetectable at this level of sampling) across the central Gulf Coast. If east-west migration is occurring or has occurred in the recent past, we probably would have identified at least one derived Florida haplotype in a mottled duck from Texas or Louisiana or vice versa. But, no derived haplotypes are shared across the geographic boundary. Instead, we see a clade of haplotypes endemic to Florida and a clade of haplotypes endemic to Texas and Louisiana, and these account for 52.5% of mottled ducks sampled in our study (Figures 2, 3). Substantial hierarchical structure within these clades, and divergence between them, suggests that this pattern is not a recent development, but that these two groups of haplotypes have been isolated for many generations. For example, the Florida haplotype group is strongly diverged, showing six to eleven informative nucleotide substitutions relative to any haplotype in the Texas/Louisiana group. In contrast, no more than four informative nucleotide substitutions differentiate any two members of the Texas/Louisiana group. The fact that Florida and Texas/Louisiana haplotypes are reciprocally monophyletic and coalesce to a common ancestral haplotype, furthermore, suggests that geographic isolation may not always have existed. Perhaps a formerly panmictic population was subdivided by vicariant climate or habitat change on the central Gulf Coast?

The remaining 47.5% of mottled ducks sampled in our study possess haplotypes that branch basally, show significantly less hierarchical structure, and fall into closely related lineage groups of mixed species composition. In the absence of an obvious geographic pattern, the relationships of these haplo-

types suggest that incomplete lineage sorting or ongoing/past hybridization (or some combination of both) are responsible for the pattern observed in this part of the tree (e.g., Avise et al. 1983, 1990; Ferris et al. 1983; Tegelstrom 1986; Cann et al. 1987). To the extent that Mexican ducks, black ducks, or mallards are close relatives of mottled ducks, patterns of mixed species composition observed in the basal part of our tree are consistent with oft proposed pattern that results from incomplete matriarchal lineage sorting from a polymorphic ancestral gene pool (e.g., Tajima 1983; Neigel and Avise 1986; Nei 1987; Avise et al. 1990). Such patterns are not uncommon in species or populations that have separated recently relative to their effective population sizes. In the case of mottled ducks, approximately 47.5% of sampled individuals possess one of many putative "ancestral" haplotypes, whereas the remaining 52.5% possess the derived mottled duck haplotypes.

Ongoing and past hybridization with other members of the New World mallard group also can result in an indistinguishable pattern of haplotype relationships. For example, the two mallards sampled in the Central Flyway that occur in the clade of haplotypes believed to be endemic to mottled ducks in Texas and Louisiana (Figure 2; see above) could have acquired mottled duck mitochondria via male mallard $\times$ female mottled duck hybridization. Mottled ducks with additional white feathers in the speculum show no particular association with any given clade or group of haplotypes (see Figures 2, 3). However, six to ten times as many Florida and Peach Point WMA mottled ducks showed hybrid characters as did mottled ducks collected elsewhere in Texas and Louisiana, and several mottled ducks possessed haplotypes identical or nearly identical to haplotypes found in Mexican ducks, black ducks, and mallards (Figures 2, 3). It is well-known that black ducks and mallards share similar, and in some cases, identical mitochondria haplotypes (Avise et al. 1990; see also Ankney et al. 1986; Patton and Avise 1986), and the same may be true for mottled ducks and Mexican ducks.

Mottled duck population genetics

Pairwise F_{ST} values for Florida versus all other localities (Table 3) confirm the pattern evident in the haplotype tree (Figures 2, 3). Despite estimated levels of shared ancestral polymorphism or hybridization of 27.3% for Florida and 51.1% for Texas/Louisiana, F_{ST} values for all comparisons including Florida are highly significant (all Ps < 0.0001). These results are consistent with data from banding records. Between 1922 and 1998, 42,369 mottled ducks were banded in the United States, and 4,240 (10.0%) of these have been recovered (U.S. Geological Survey, Biological Resources Division, Patuxent Wildlife Research Center, Laurel, Maryland). Of these, 2,811 (66.3%) were banded in Texas and Louisiana, and 1,273 (30.0%) were banded in Florida. No mottled ducks banded in Florida have been recovered in Texas or Louisiana or vice versa. Most of the remaining 156 (3.7%) recovered mottled ducks originated from release programs and were banded in South Carolina; one of these birds was recovered in Florida.

Nucleotide mismatch distributions suggest that, as a species group, the four New World mallard species have experienced a recent population expansion (Figure 4a). North American mallard species are relatively recently evolved lineages of a species complex that is distributed worldwide (Livezey 1991; Johnson and Sorenson 1999; see below). As a result, closely spaced divergence dates have allowed relatively little time for maternal lineages to sort relative to their effective population sizes. In contrast, mismatch distributions for Florida and Texas/Louisiana (Figure 4b) are multi-modal and congruent with the highly structured pattern evident for derived mottled duck haplotypes in Figure 2. Florida mottled ducks probably were once more numerous than they are today given recent urban and agricultural pressures.

Relationships of mottled ducks to other mallard species

Mottled duck haplotypes identified in our study show close phylogenetic relationships to haplotypes identified in each of the other three New World mallard species, including the Mexican duck, black duck, and mallard (Figures 2, 3). In several instances, the haplotypes are identical. Thus, an investigation of the relationships of mottled ducks to other New World mallard-like species is not only interesting, but vital to our understanding of the population biology of mottled ducks. Some haplotype similarities probably result from introgression of the mitochondria across species boundaries, whereas others probably result from common ancestry. To tease these processes apart we pose two questions: (1) what are the mottled duck's closest relatives, and (2) what are the phylogenetic relationships of the mallard group?

Many authors (e.g., Delacour 1956; Johnsgard 1961, 1965; Kear 1970; Bellrose 1976; Kear and Murton 1976; Palmer 1976; Graham 1979; Weller 1980; Ankney et al. 1986; Ankney and Dennis 1988; Hepp et al. 1988; Avise et al. 1990; Livezey 1991, 1993; Rhymer et al. 1994; Cooper et al. 1996; Peterson 1996; Omland 1997; Johnson and Sorenson 1999; Sorenson et al. 1999; Rhymer 2001) have tried to answer these and other questions related to mallard relationships. Avise et al. (1990) were among the first to investigate these questions using modern molecular techniques (mtDNA RFLP analysis). They described two divergent, yet sympatric clades of mallard haplotypes, one shared only by mallards (type A haplotype) and the other shared by mallards and black ducks (type B haplotype). Based on these findings, Avise et al. (1990) concluded that black ducks and mallards are closely related. More specifically, they proposed that black ducks evolved recently and rapidly at the periphery of the mallard range in eastern North America. As such, mallards are paraphyletic, and black ducks evolved by peripatric speciation. Avise et al. (1990) proposed incomplete matriarchal lineage sorting from a polymorphic ancestral gene pool as the explanation for mallard paraphyly. Although Avise et al. (1990) did not include mottled ducks or Mexican ducks, they speculated that mottled ducks, like the black duck, are a sexually monochromatic form of the mallard (see also Bellrose 1976; Graham 1979; Ankney et al. 1986).

However, Avise et al. (1990) recognized that mallard paraphyly also might be explained by the acquisition of black duck mitochondria by mallards via hybridization with female black ducks, but they dismissed this possibility as improbable, given the current range of black ducks in northeastern North America. At the time no estimate of mallard phylogeny was available to Avise et al. (1990), but new estimates of phylogeny for twelve of the world's fourteen extant or recently extinct mallard-like species (Johnson and Sorenson 1999; see also Livezey 1991) now strengthen the case for introgression of nominate mallards into the New World species group, rather than peripatric speciation. Johnson's and Sorenson's (1999) ND2 and cytochrome *b* data, including 2,166 nucleotide positions, yields an estimate of mallard phylogeny that is more consistent with inter-specific hybridization (Figure 5). Within this tree, mallards show two mitochondrial haplotypes that differ by 0.58% sequence divergence. One of these haplotypes is identical to haplotypes found in black ducks and Mexican ducks and nearly identical (2 nucleotide differences) to haplotypes found in mottled ducks, (Avise et al.'s (1990) type B, our ingroup haplotypes), whereas the other haplotype (Avise et al.'s (1990) type A, our outgroup haplotypes) differs at a single position from an Asian/Pacific clade, which includes the Indian spot-billed duck (*A. poecilorhyncha*), Chinese spot-billed duck (*A. zonorhyncha*), and Phillipine duck (*A. luzonica*) (see also Johnson and Sorenson 1999). With the exception of the mallard haplotype identical to black duck and Mexican duck haplotypes, the tree shows a simple biogeographic pattern. Mallard species originated in the Southern Hemisphere, probably Africa, followed by step-wise migration to the South Pacific and Asia and a single migration to the New World. If Avise et al.'s (1990) explanation of incomplete lineage sorting is correct, then additional mallard haplotypes should fall in alternative positions on the tree depicted in Figure 5, but this is not the case as noted by Johnson and Sorenson (1999). If mallards originated in the New World, invaded the Old World, and hybridized with the Indian spot-billed duck, then one haplotype group (the ancestral haplotypes) should occur in the New World and two haplotype groups (the ancestral haplotypes plus the hybrid haplotypes) should occur in the Old World, but this also does not appear to be the case. On the contrary, two haplotype groups occur in the New World, whereas only one haplotype group occurs in the Old World (Johnson and Sorenson 1999; K.G. McCracken, S. Rohwer, and K.S. Winker unpubl. data). The only explanation that fits these patterns is that nominate mallards originated in the Old World, probably in Asia, subsequently invaded the New World, and in the process acquired black duck mitochondria via hybridization (see Palmer 1976; Johnson and Sorenson 1999 for further discussion). Contrary to the prevailing opinion, black ducks probably diverged from a monochromatic mallard-like ancestor that migrated from the Old World and established itself in the New World well in advance of nominate mallards.

Our data bear clearly on the question of mallard paraphyly, and the relationship of the mottled duck to other mallard species. First, our data suggest that hybridization with mallards is not limited to black ducks (Goodwin 1956; Johnsgard 1960, 1967; Heusmann 1974; Brodsky and Weatherhead 1984), but also occurs with mottled ducks (at least two instances in our data), and probably Mexican ducks (Bellrose 1976; Graham 1979). The fact that 47.5% of mottled ducks, 100% of Mexican ducks, 100% of

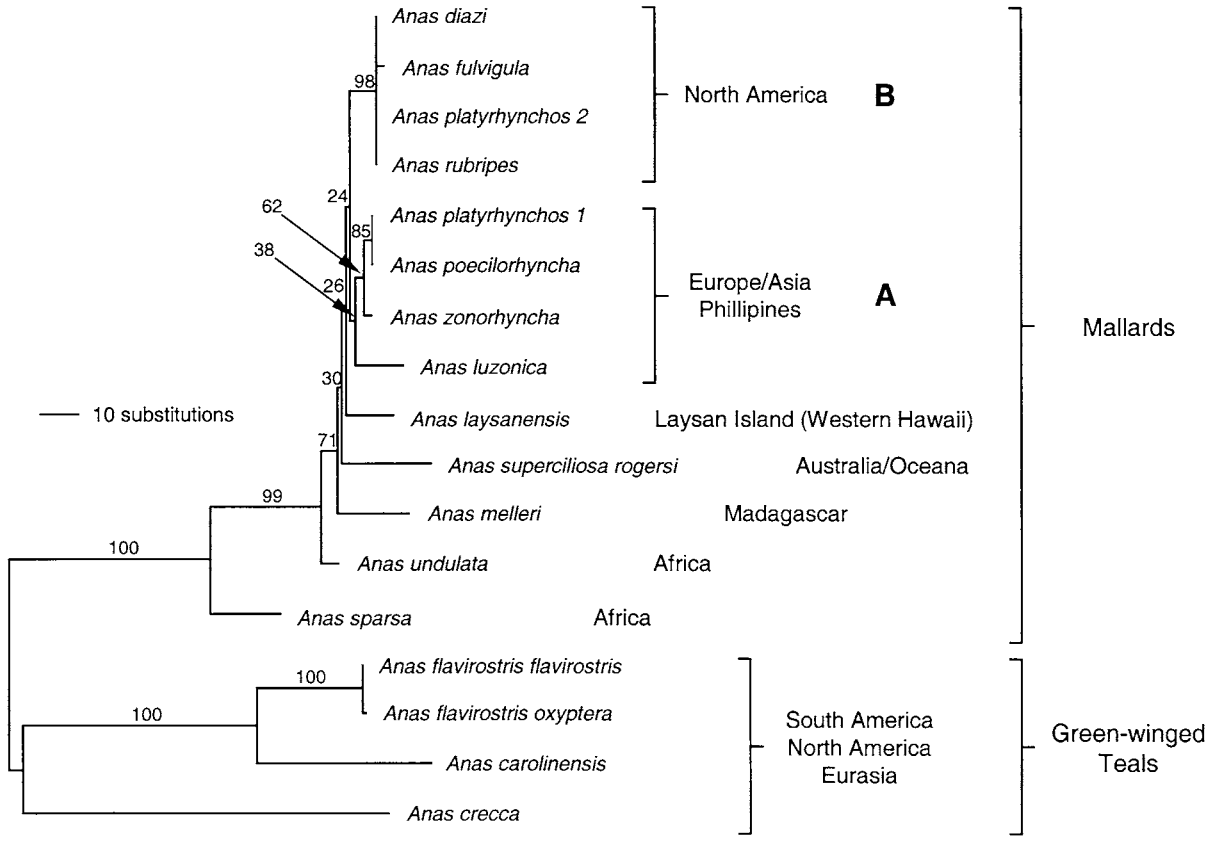

Figure 5. Phylogenetic and area biogeographic relationships of the mallard group rooted on the green-winged teals. Maximum likelihood tree based on 2166 nucleotide positions in the ND2 and cytochrome *b* genes (Johnson and Sorenson 1999; lnL = −4641.06 ti:tv = 16.15, p-inv = 0.79). Bootstrap consensus indices (1000 replicates) indicate support for nodes. Bold letters A (our outgroup haplotype) and B (our ingroup haplotype) correspond to haplotypes described by Avise et al. (1990); Johnson and Sorenson (1999) described these haplotypes as 1 and 2.

black ducks, and 50% of mallards sampled in our study fall into closely related lineage groups of mixed species composition, likewise, can be interpreted as incomplete lineage sorting of a polymorphic ancestral gene pool reshuffled by hybridizing mallards. Avise et al. (1990) previously applied incomplete lineage sorting criteria to explain haplotype identity between mallards and black ducks, but we believe the lineage sorting process is correctly applied to mottled ducks, Mexican ducks, and black ducks. If the hybridization scenario is correct, black ducks, mottled ducks, and Mexican ducks are the descendants of the first monochromatic mallard-like species to inhabit North America (e.g., Palmer 1976). The plumage similarities are obvious; nominate mallards are the exception to the rule. The position of the single Mexican duck haplotype (collected from four individuals well within the interior of Mexico fifty years ago) in our tree (Figure 2) suggests that Mexican ducks may be closest to mottled ducks, and that the two non-migratory species are nested within black ducks. Mild climate and year-round food resources are just two factors that might have led mottled ducks and Mexican ducks to diverge in the subtropics of North America.

One caveat of our conclusion is that black ducks formerly might have been much more widespread across North America than they are today. Hybridization also may be largely completed. If these hypotheses are true, two of the three reasons Avise et al. (1990, p. 1115) rejected the hybridization scenario are untenable. The third reason Avise et al. (1990) rejected the hybridization argument applies to the direction of the cross. As pointed out by Avise et al. (1990), male mallards typically are dominant to male black ducks and commonly displace them when competing for females of either species (Johnsgard 1960; Brodsky and Weatherhead 1984; Brodsky et al. 1988; but see D'Eon et al. 1994; Hoysak and Ankney 1996;

McAuley et al. 1998). However, we believe Avise et al. (1990) mistakenly identified the type B haplotypes as originally characteristic of some mallards and argue, instead, that the B haplotypes were originally characteristic of black ducks. As a result, hybrids are expected to acquire black duck mitochondria (Avise et al's (1990) type B haplotype) preferentially, and when back-crossed to pure mallards, black duck mitochondria spread through the mallard population. Hybridization of this kind between mallards and black ducks probably explains why Avise et al. (1990) were not able to distinguish the date of "separation" of black ducks from mallards in the same lineage from zero time before present; mallard and black duck type B haplotypes are effectively identical by descent due to ongoing flow of genetic material across species boundaries.

The hybridization hypothesis can be tested further. If Avise et al.'s (1990) type B haplotype (our ingroup haplotype) is discovered in mallards native to Asia, and mallards carrying the black duck mitochondria have not re-invaded Asia secondarily, the hybridization argument may be falsified. On the other hand, east-west variation in black duck and mallard haplotype frequencies across North America and Asia might reveal a relict signature of the hybridization event if ancestral haplotype frequencies have not been swamped by hybridization. A population genetics study, such as that developed here for mottled ducks, for the Old World mallard group would be of benefit, as would a study of Mexican ducks in the interior of México and the southwest United States where mallards and Mexican ducks are sympatric. Segregating nuclear markers, in particular, could identify individuals of hybrid ancestry.

Conservation and management of mottled ducks

It is not surprising that a strong geographic element exists in the genetic structure of mottled duck populations, as similar phylogeographic patterns exist in many other species endemic to southeastern North America (e.g., Swift et al. 1986; Avise and Nelson 1989; Walker and Avise 1998). As a year-round resident of the Gulf Coast and one of two non-migratory dabbling ducks that inhabit North America, the mottled duck's population biology naturally lends itself to such structuring. As to whether similar population structuring appears in Mexican ducks or other subtropical North American waterfowl remains open to question. Identification of a strong geographic element in the meta-population structure of mottled ducks has important consequences for conservation of this species across its entire range. First, conservation of mottled ducks in peninsular Florida should be a high priority, given the relatively small size of the Florida population (~56,000; Johnson et al. 1984; Brust 1993) and the large number of endemic haplotypes we identified in our study; 72.7% of mottled ducks sampled in Florida possessed haplotypes endemic to Florida. However, the relatively small size of our sample in Florida suggests that more sampling may identify greater haplotype diversity. Growing urbanization, agricultural pressure, and hybridization with feral mallards make conservation concerns in Florida all the more compelling (Moorman and Gray 1994). Current estimates indicate that there are 500,000–800,000 mottled ducks in Texas and Louisiana (B. Wilson, Gulf Coast Joint Venture unpubl. data), yet mottled ducks in Louisiana and Texas still face threats such as loss and degradation of wetland habitat (Michot 1996). Unlike Florida, hybridization with mallards does not appear to be an immediate concern in Texas or Louisiana; however, evidence concerning hybrid pairing is scarce (Stutzenbaker 1988). Paulus (1988) observed that eight of 225 mottled duck pairs in Louisiana involved mallards; four female mottled ducks were with mallard males, and four male mottled ducks were paired with female mallards. Even so, hybridization is still a factor that could threaten mottled ducks in Texas and Louisiana. Additionally, many counties along the Texas coast are experiencing rapid population growth (Ramos 1999), and populations of feral mallards will likely undergo a concomitant increase. The fact that mottled ducks in Texas/Louisiana and in Florida are evolutionary distinct only heightens the need to be concerned about hybridization. Should mottled duck populations in one of these regions decline to the point where restrictive harvest measures or season closures are warranted, we see no reason why the population inhabiting the other region could not continue to be managed and harvested independently.

Taxonomy for the new world mallards

Many authors, including the American Ornithologists' Union (1998), have considered the mottled duck and Mexican duck to be monochromatic sub-species or semi-species of the mallard (e.g., Peters 1931; Bellrose 1976; Johnsgard 1978; Sibley and Monroe 1990).

However, our data and Johnson's and Sorenson's (1999) data are more consistent with the idea that mottled ducks and Mexican ducks are each others closest relatives and in turn most closely related to black ducks. Both mottled ducks and Mexican ducks mate assortively (Aldrich and Baer 1970; Bevill 1970; Paulus 1988), and a large clade of derived mottled duck haplotypes indicates that lineage divergence has occurred historically. To the extent that these findings are corroborated, we recommend that mottled ducks and Mexican ducks be designated as species so that the nomenclature is consistent with phylogeny.

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