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Low Mitochondrial DNA Variation in Double-crested Cormorants in Eastern North America

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Abstract.—Double-crested Cormorant (*Phalacrocorax auritus*) numbers are increasing throughout eastern North America. We compared variation for five portions of mtDNA to determine if genetic differences existed among portions of the breeding range that would need to be considered when formulating management programs. Sequences for four mtDNA regions were identical across sample locations; frequencies of two haplotypes of the mitochondrial Control Region were similar across sampling locations. There is no evidence of restricted gene flow among breeding areas, or between subspecies with different migratory patterns. Received 6 June 2002, accepted 25 November 2002.

Key words.—Double-crested Cormorants, *Phalacrocorax auritus*, mtDNA, aquaculture, subspecies, migration.

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The Double-crested Cormorant (*Phalacrocorax auritus*) is an abundant piscivore found across much of North America. Destruction of rookery sites, shooting, and reduced nest success from pesticide exposure were apparently responsible for a large decline in abundance of cormorants in the 19th and early 20th Centuries (Hatch 1995). This species was protected in 1972 under the United States-Mexican Convention for the Protection of Migratory Birds and Game Mammals (Trapp *et al.* 1995). Recently, numbers have expanded to historically high levels. In 1990, 1-2 million individuals were estimated to inhabit North America (Hatch 1995). This increase in numbers has been greatest in traditional breeding areas of the northeast and upper midwest of the United States and the maritime and prairie provinces of Canada (i.e., Chapdelaine and Bédard 1995; Krohn *et al.* 1995; Weseloh *et al.* 1995). Associated with this increase in numbers of breeding birds is a large increase in the number of individuals wintering in the southeastern United States (Brugger 1995; Hatch 1995; Jackson and Jackson 1995).

The recent increases in abundance and calls for control have resulted in a review of the status of the Double-crested Cormorant by the U.S. Fish and Wildlife Service (2001).

Management decisions are hampered by lack of knowledge of the genetic structure in this species (Hatch and Weseloh 1999). There is considerable genetic structure in the Great Cormorant (*P. carbo*; Goostrey *et al.* 1998), raising the possibility that Double-crested Cormorants from different portions of the breeding range may also exhibit genetic differences.

Genetic structure could be of management concern because of the existence of two subspecies of Double-crested Cormorant in eastern North America with different migratory patterns. East of the Continental Divide, breeding colonies located in the northern U.S. and southern Canada and are classified as *P. a. auritus* (Lesson, 1831). This subspecies is strongly migratory (Bent 1922; Hatch 1995). On the basis of banding data (Kury and Cadbury 1970; Dolbeer 1991; Brugger 1995; Hatch 1995), the birds winter in the southeastern United States from North Carolina to Texas. Other breeding colonies, allopatric with *P. a. auritus*, occur in Florida and, to a lesser extent, in surrounding states (Brugger 1995; Hatch 1995; Reinhold *et al.* 1998; Hatch and Weseloh 1999). These birds are classified as *P. a. floridanus* (Audubon 1835), and individuals nesting in Florida are considered to be non-migratory

(Bent 1922; Hatch 1995; Hatch and Weseloh 1999). Hatch (1995) and Hatch and Weseloh (1999) consider individuals nesting in Florida and the northern United States to be different populations. In addition to the differences in migratory patterns and the allopatry of the breeding sites of the two taxa, morphological analyses indicate that the Florida subspecies is smaller than *P. a. auritus* (Watson *et al.* 1991; Hatch 1995). It is unknown whether these subspecies, which have been distinguished on the basis of morphology and migratory behavior, are genetically differentiated. If there are large genetic differences between the subspecies, the non-migratory *P. a. floridanus* might warrant special consideration for protection as evolutionary significant unit (Moritz 1994a). However, if migratory birds occasionally remain in the south to nest with resident birds, gene flow between the putative subspecies might be high enough to prevent genetic differentiation.

Mitochondrial DNA has been used to study phylogeographic relationships among populations of many species of animals (Awise 1994; Moritz 1994b). Our objective was to determine if mtDNA variation exists within and between the two subspecies of Double-crested Cormorants inhabiting the eastern United States and Canada.

METHODS

To determine the extent of variation within or between subspecies, we initially sequenced DNA from three individuals sampled from each of three geographic regions, Florida, the Northern Prairie (North Dakota and Saskatchewan), and Great Lakes—St. Lawrence Seaway (Ontario and Quebec). Blood samples were collected from pre-fledgling birds during 1998-2000 (Fig. 1); investigators contributing blood were asked to sample only one individual per nest. DNA was isolated from these samples with Gentra's Puregene DNA Isolation protocol for whole blood with 7.5 M ammonium acetate for DNA precipitation and resuspension in dH₂O.

Five regions of the mtDNA were sequenced. Ribosomal RNA primers 12L5 and 12H4 were obtained from Hedges to examine the 12S rRNA region (1994). The 16S rRNA was amplified with primers 16L2 and 16H13 (Hedges 1994); 16H12 was used as an internal sequencing primer (Hedges and Sibley 1994). Cytochrome *b* L14841 and H15149 primers and control region primers L15926 and H00651 were obtained from Kocher *et al.* (1989). The CO3-ND3 region was sequenced using primers L10647 and H11100 (Mindell *et al.* 1998). Amplifications and sequencing of fluorescently-labeled am-



Figure 1. Geographic locations (dots) of individuals used to examine the mtDNA variation of the Double-crested Cormorant (*Phalacrocorax auritus*). FL1—St. Martin's Keys, Citrus Co. (N28°49, W82°39); FL2—Lake Kissimmee, Osceola Co. (N28°47, W81°03); FL3—Cocoa Beach, Brevard Co. (N28°19, W80°36); FL4—Sanibel Island, Lee Co. (N26°26, W82°06); ND—Chase Lake (N47°00.70, W99°27.20); SAS—Dore Lake (N54°29, W108°16); QUE—St. Lawrence River (N70°36, W47°23); ONT—Steamboat Island, Lake Superior (N46°39, W84°36).

plification products were performed using AmpliTaq Gold polymerase and standard protocols provided by the manufacturer (Applied BioSystems, ABI). Sequences, generated on an ABI 310 Genetic Analyzer, were aligned with published avian mtDNA. Aligned sequences were translated into amino acid sequences; no premature stop codons were found in coding regions.

RESULTS

No variation was found in the 12S rRNA, 16S rRNA, CO3-ND3, and cytochrome *b* sequences obtained from the three locations we sampled (Table 1). However, a single base insertion was found among sequences of the mitochondrial control region (i.e., D-loop) of the mtDNA. This region was sequenced for nine additional individuals, including individuals from sites in Florida that were not analyzed previously, to assess the distribution of the two haplotypes (Table 1, Fig. 1). GENEPOP was used to examine variation in

Table 1. Sequences examined for Double-crested Cormorant mtDNA for three portions of the breeding range. Numbers of individuals with the same sequence are listed by sampling region, along with the accession number of sequences deposited in Genbank.

Sequence	Fragment length ¹	Numbers of individuals sharing sequence			Genbank Accession Number
		Florida	Northern Prairie ²	Great Lakes-St. Lawrence Seaway ³	
12s RNA	414	3	3 (2 from ND, 1 from SAS)	3 (from QUE)	AF373588
CO3-ND3	461	3	3 (2 from ND, 1 from SAS)	3 (2 from QUE, 1 from ONT)	AF373589
Cytochrome b	342	3	3 (3 from ND)	3 (1 from QUE, 2 from ONT)	AF373590
16s RNA	992	3	3 (2 from ND, 1 from SAS)	3 (2 from QUE, 1 from ONT)	AF373591
Control region					
Haplotype A	355	4	2 (1 from ND, 1 from SAS)	4 (2 from QUE, 2 from ONT)	AF373592
Haplotype B	356	2	4 (2 from ND, 2 from SAS)	2 (1 from QUE, 1 from ONT)	AF373593

¹Number of bases in the sequenced fragment.

²Individuals are from North Dakota (ND) or Saskatchewan (SAS).

³Individuals are from Ontario (ONT) or Quebec (QUE).

haplotype distribution in the three geographic samples. There was no apparent difference in the frequency of the two control region haplotypes among samples ($P = 0.59$) based on randomization test from GENEP-OP (Raymond and Rousset 1995). Both variants occurred at a high frequency in each sampling location (Table 1).

DISCUSSION

Little variation was found in five regions of mtDNA sequenced for samples from either subspecies. While it was not surprising that conservative regions of the mtDNA such as the CO3-ND3, 16S and 12S exhibited no differentiation among individuals, some of these slowly evolving regions have exhibited sequence differentiation among disjunct populations of other taxa (e.g., Matthee and Fleming 2002; Paulo *et al.* 2002). More striking is the lack of differentiation between the two subspecies at the cytochrome *b* and control regions. These regions of the mtDNA evolve relatively rapidly and have been used to detect population structure in many other avian species (e.g., Wenink *et al.* 1996; Buerkle 1999; Kirchmann *et al.* 2000; Burg and Croxall 2001).

One reason for a lack of genetic differentiation might be high gene flow, but this is difficult to assess because there is little variation in the regions of mtDNA we examined. With the limited sample sizes used in this

study, it would be difficult to detect anything other than large shifts in haplotype frequencies. However, sample sizes were sufficient to identify major phylogenetic subdivisions if they had existed. Widespread distribution of both of the control region haplotypes provides stronger evidence that there is gene flow among sampling locations, this could be due to recent population expansion in the last 30 years. While most of the expansion in numbers has occurred in traditional breeding areas, a few colonies have become established between the summer ranges of the two subspecies (Hatch 1995). One such nesting aggregation occurs in North Carolina where individuals are intermediate in morphology between *P. a. auritus* and *P. a. floridanus*, suggesting the possibility of gene flow (Hatch 1995). Banding data shed little light on the amount of gene flow among *P. a. auritus* and *P. a. floridanus*. Movement studies have shown that individuals do not maintain roost-site specificity in wintering areas (King 1996) and they spread out over large geographic areas when migrating north to breed (Dolbeer 1991; Brugger 1995). Although banding returns indicate that adults often nest near their natal colonies, several instances of long distance dispersal to other breeding colonies have been observed (Dolbeer 1991).

If *P. a. floridanus* was isolated from the migratory *P. a. auritus* for a prolonged period, sequence divergence would be expected. Buerkle (1999) found substantial divergence

between migratory Prairie Warblers (*Dendroica discolor*) nesting in the northern United States and non-migratory populations in Florida, suggesting that the migratory and non-migratory forms have been isolated since the late Pleistocene. Unless rates of mtDNA evolution in warblers and cormorants are dramatically different, non-migratory *P. a. floridanus* must have been isolated much more recently than the Pleistocene, and raise the possibility that gene flow is ongoing.

This study provides no evidence to justify managing *P. a. floridanus* differently than *P. a. auritus* or for treating them as separate subspecies. It is possible that gene flow between the subspecies is large and that differences in size of cormorants from Florida and northern individuals is due partially to environmental influences on growth, as has been documented in other birds (James 1983).

Subspecies are expected to exhibit genetic differentiation (Mayr 1963, O'Brien and Mayr 1991; Ball and Avise 1992). While differences in mtDNA would have been strong support to the taxonomic distinction of *P. a. floridanus* and *P. a. auritus*, the lack of sequence differentiation makes this taxonomic distinction ambiguous (Ball and Avise 1992). The mtDNA sequences we examined are all part of one non-recombining genetic unit and it is possible that independent nuclear genes would exhibit genetic differentiation concordant with the geographic ranges of the two taxa. Given the limited amounts of mtDNA variation that were detected, our understanding of the taxonomic status of these putative subspecies would benefit from additional analyses with more polymorphic nuclear genetic markers.

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