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SHORT COMMUNICATION

Matrilineal history of the endangered Cape Sable seaside sparrow inferred from mitochondrial DNA polymorphism

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Abstract

Restriction analyses were conducted on mitochondrial DNA (mtDNA) amplified by long-PCR from an endangered bird, the Cape Sable seaside sparrow. The first of several successful mtDNA amplifications was accomplished using the partially digested tissue remains of a transmitter-monitored bird retrieved from the gut of a snake. As many as 91 mtDNA restriction fragments produced by 18 endonucleases were compared in this and four other Cape Sable specimens against mtDNA similarly amplified by long-PCR from other taxonomic forms in the seaside sparrow complex. Results indicate that the Cape Sable birds belong to an 'Atlantic' matrilineal clade, and are highly divergent from other seaside sparrows along the Gulf of Mexico.

Keywords: conservation genetics, long-PCR, phylogeography, restriction analysis, RFLPs

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Introduction

The entire range of the Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*) is confined to extreme southwestern Florida (Fig. 1), primarily in the Everglades National Park where several thousand birds continue to exist (Ehrlich *et al*. 1992). This Cape Sable population is part of a taxonomic assemblage of seaside sparrows (Quay *et al*. 1983) inhabiting coastal marshes of the eastern United States from New England to Texas (Fig. 1). The Cape Sable population was described originally as a distinct species (Howell 1919), purportedly the last avian species to be discovered in the continental U.S. Later, the Cape Sable population was demoted to subspecific status (Eisenmann 1973) within the seaside sparrow complex. Due to its narrow distribution and attendant vulnerability to catastrophic natural disasters, the Cape Sable seaside sparrow was placed on the original Federal list of endangered species in 1967, where it remains today.

An earlier study of mitochondrial DNA (mtDNA) in six geographical subspecies of seaside sparrow identified two highly distinct matriarchal assemblages within the complex, one confined to the Gulf of Mexico, the other to the Atlantic coast (Fig. 1; Avise & Nelson 1989). This genetic finding was consistent with an earlier biogeographic scenario, based primarily on geological evidence, that the species had been sundered historically into 'Atlantic' and 'Gulf' units, perhaps during the Pleistocene (Funderburg & Quay 1983). However, the conventional taxonomic assignments upon which management programmes for the seaside sparrow (Quay *et al*. 1983) have been based do not reflect this salient phylogeographic subdivision (Avise & Nelson 1989; Avise 1989).

Here we examine the matrilineal history of the endangered Cape Sable seaside sparrow by comparing its mtDNA sequences to those reported previously for other named subspecies in the complex. With respect to mtDNA, three distinct and testable outcomes are possible: each specimen of the Cape Sable seaside sparrow could be allied closely to (i) the Atlantic matrilineal clade; (ii) the Gulf matrilineal clade; or (iii) neither. Results should be relevant to management efforts directed toward this 'endangered species.'

Materials and Methods

Tissue samples

Permit applications to sacrifice a few sparrows for genetic analysis were denied, so tissues had to be obtained by

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Fig. 1 Nine traditionally recognized subspecies of seaside sparrow (after Avise & Nelson 1989). Black ellipses indicate the ranges of subspecies that proved to belong to the 'Atlantic' matrilineal clade; open ellipses are the ranges of assayed subspecies belonging to the 'Gulf' matrilineal clade. The subspecies *pelonota* and *sennetti* have not yet been assayed genetically. The endangered form *mirabilis* is the novel focus of the current study.

other means. The first specimen for the current study was extracted from the stomach of a cottonmouth snake (*Agkistrodon piscivorus*) who in nature had killed and eaten the bird within the previous 24 h. Discovery of the unhappy fate of this bird and the retrieval of its partially digested carcass were possible because the bird had been fitted with a radio transmitter as part of a dispersal study funded by the U.S. Fish and Wildlife Service, National Park Service, and the Army Corps of Engineers. From this individual, mtDNA was isolated from liver tissue. From four other Cape Sable specimens captured by wildlife personnel, small samples of blood (30–50 μL) drawn from the brachial vein were the biological source for mtDNA amplifications.

Laboratory protocols

From 'fresh' liver of the snake-retrieved specimen, DNA was isolated by CsCl gradient centrifugation (Lansman *et al*. 1981), albeit in insufficient quantities for direct analysis by restriction enzyme digestions. From blood of the other samples (stored in a lysis buffer consisting of 100 mM Tris-HCl, 100 mM EDTA, 10 mM NaCl, and 1.0% SDS), DNA was extracted by a standard phenol/chloroform/isoamyl alcohol procedure, followed by ethanol precipitation. The resulting DNA was resuspended in 250 μL TE buffer.

Procedures for primer design and long-PCR generally followed those detailed in Nelson *et al*. (1996), with the following modifications for the current study. Using universal primers 16sar-L and 16sbr-H (Palumbi *et al*. 1991), the 16S ribosomal gene was amplified from each of five Cape Sable specimens. Negative controls were employed to ensure that no contamination had occurred. The resulting amplification products were cleaned via the Wizard PCR Preps DNA Purification System (Promega). Heavy and light strands of the amplified fragments were cycle-sequenced using the Promega fmol DNA Cycle Sequencing System. Sequences were aligned and the following 32-bp primers were designed specifically from the seaside sparrow's 16S ribosomal gene: DS-for-long (5'-GGGGAGGTTTACGACCTCGATGTTGGATCAGG-3'); DS-rev-long (5'-TGATTGCGCTACCTTTGCACGGTTAGGATACC-3').

These primers, roughly 500 bp apart, were used to amplify nearly full-length mtDNA from the Cape Sable sparrows, as well as from other representative seaside sparrows assayed in the original study by Avise & Nelson (1989), using the Hybaid Thermal Cycler and the Expand Long Template PCR system (Boehringer-Mannheim). Optimized conditions, achieved according to the manufacturer's recommendations, were carried out in 50 μL reaction volumes: 1× buffer 3, 2.5 units enzyme mix, 100 ng DNA, 350 μM of each dNTP, and 25 pmol of each

long-PCR primer. Cycling parameters consisted of an initial denaturation at 94 °C for 2 min, 35 cycles at 92 °C for 15 s, annealing at 48 °C for 15 s, and extension at 68 °C for 12 min. Negative controls again were employed as a check against contamination.

To verify amplification initially, 5 μL of each reaction mixture was electrophoresed through a 2% agarose gel which was then stained with EtBr. Subsequent restriction assays used the same suite of 18 enzymes that had been employed in the earlier survey of seaside sparrow mtDNA (Avise & Nelson 1989). The mtDNA digestion profiles were revealed by EtBr staining of agarose gels, with fragment sizes compared against a 1-kb ladder standard.

Results

About 97% of the mtDNA molecule was amplified by the long-PCR primers. Because these amplification products are slightly shorter than full-length mtDNA, and are linear rather than closed-circular, their restriction digestion profiles differ accordingly from those of whole native mtDNA. For example, the *Pst*I pattern 'C' in earlier digestions of closed-circular mtDNA (Avise & Nelson 1989) displayed three fragments on gels of approximate sizes 8.8, 6.5, and 1.2 kb. In comparable digests of the long-PCR products in the current study, four *Pst*I gel bands appeared of approximate lengths 8.8, 3.4, 2.6, and 1.2 kb. Thus, the two long-PCR priming sites must have fallen near the centre of the original 6.5 kb fragment such that it (minus the small nonamplified region of the 16S ribosomal gene) was converted to the 3.4 and 2.6 kb fragments in the restriction digests of the long-PCR products.

For the snake-retrieved specimen of the Cape Sable seaside sparrow analysed following long-PCR, all 18 enzymes originally employed by Avise & Nelson (1989) again produced readily scorable mtDNA digestion patterns (example in Fig. 2). In that earlier study, five of the 18 restriction enzymes cleanly distinguished (sometimes by more than one restriction site) the Atlantic and Gulf matrilineal phylogroups (Table 1). As gauged by the total of 91 restriction fragments scored from this first Cape Sable specimen, its mtDNA matched perfectly the most common mitochondrial genotype previously reported for the Atlantic matrilineal clade (Table 1; Fig. 2).

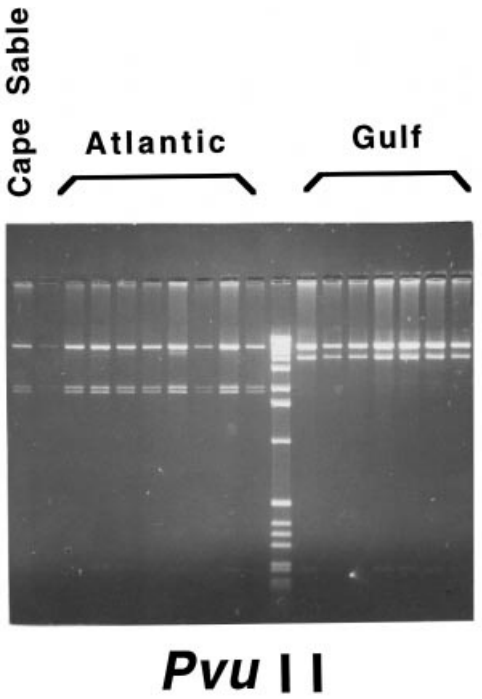

Fig. 2 Gel profiles produced by *Pvu*II digestion of mitochondrial DNA (mtDNA) amplified by long-PCR. The eighth lane from the right is a 1-kb molecular size standard. Specimens to the right of this standard display the mtDNA profile characteristic of Gulf coast birds; those to the left display the characteristic Atlantic genotype. The leftmost lane represents the specimen of the Cape Sable seaside sparrow retrieved from the snake's stomach.

Common Atlantic genotype†:	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*	*C*
Common Gulf genotype†:	C	*G*	*D*	C	C	C	C	*D*	C	C	*D*	C	C	*G*	C	C	C
Cape Sable seaside sparrow #1:	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Cape Sable seaside sparrow #2:	C		C				C							C			
Cape Sable seaside sparrow #3:	C	C	C	C	C		C							C			
Cape Sable seaside sparrow #4:	C	C	C		C		C							C			
Cape Sable seaside sparrow #5:	C	C	C		C		C										

Table 1 Mitochondrial DNA (mtDNA) genotypes in seaside sparrows revealed by 18 restriction enzymes (letters, from left to right, refer to multifragment mtDNA profiles* produced by digestion with *Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*II, *Bgl*II, *Cla*I, *Eco*RI, *Hin*cII, *Hin*dIII, *Msp*I, *Nde*I, *Pst*I, *Pvu*II, *Spe*I, *Sst*II, *Stu*I, and *Xba*I)

*For a given restriction enzyme, adjacent letters in the alphabet indicate that the digestion profiles differ by a single restriction site; nonadjacent letters differ by two or more restriction sites. Blank spaces indicate missing data. No mtDNA length variation was detected in this study.

†From Avise & Nelson (1989). Among 21 birds collected along the Atlantic Coast, 17 displayed the genotype shown here, and each of the other four specimens differed from it by a single restriction site change. Among 19 birds collected along the Gulf Coast, 14 displayed the genotype shown here, and each of the other five specimens differed from it by one or two restriction site changes.

In four other Cape Sable seaside sparrows from which blood alone was available, the long-PCR amplifications did not perform nearly as well, and only a subset of the mtDNA digestion profiles could be scored. Nevertheless, all scorable digestion patterns from these specimens were consistent with their membership in the Atlantic as opposed to the Gulf matrilineal clade (Table 1).

Discussion

Long-PCR is a recently developed technique for amplifying large DNA sequences, typically in the size range of 5–35 kilobases (Kainz *et al*. 1992; Ponce & Micol 1992; Barnes 1994; Cheng *et al*. 1994a; Cohen 1994). The method can find special application in situations where large pieces of DNA, such as a complete nuclear gene or nearly full-length animal mtDNA, are desired but otherwise may be difficult to isolate as a prelude to restriction mapping or other genetic assays (Cheng *et al*. 1994b,c; Her & Weinshilboum 1995; Nelson *et al*. 1996). Here we have employed long-PCR to amplify 16-kb sequences of mtDNA from a bird carcass retrieved from a snake's stomach, and have used restriction site data from this specimen and colleagues to evaluate the matrilineal history of an endangered avian taxon.

The snake-retrieved specimen of *Ammodramus maritimus mirabilis* belongs clearly to the 'Atlantic' rather than to the 'Gulf' matrilineal clade of seaside sparrows. So too, apparently, do the four other Cape Sable specimens assayed less completely. These findings were not necessarily anticipated because: (i) in a biogeographic treatment based primarily on historical geology and distributional evidence, Funderburg & Quay (1983) provisionally placed *mirabilis* in the presumptive clade of Gulf coast birds; and (ii) the taxonomic history (Austin 1983) and management focus (Kushlan & Bass 1983; Taylor 1983; Werner & Woolfenden 1983) on the Cape Sable seaside sparrow seems to evidence a perception that this form is unusually divergent from other seaside sparrows. To the contrary, we find that this taxonomic subspecies is extremely close genealogically to seaside sparrows along the Atlantic coast.

Strictly speaking, this conclusion applies only to the five assayed specimens. Conceivably, the Cape Sable seaside sparrow might be highly polymorphic in mtDNA with some nonassayed individuals belonging to the Gulf matrilineal clade, or perhaps even to another distinctive matriarchal phylogroup. Nevertheless, one special advantage of mtDNA analysis stems from the molecule's uniparental inheritance: each individual justifiably can be treated as an operational taxonomic unit (OTU) with respect to matrilineal ancestry (Avise 2000). Thus, whatever might be discovered about the matrilineal ties of other Cape Sable specimens, the five birds assayed would remain securely within the Atlantic mtDNA phylogroup.

The conclusion that the Cape Sable seaside sparrow belongs to the Atlantic phylogroup also applies strictly to the matrilineal component of the population's extended pedigree, and need not necessarily hold for nuclear genes. For example, one nonexcluded possibility is that the Cape Sable's nuclear genome is allied more closely to those of other seaside sparrows in the Gulf of Mexico. In theory, one way in which this discordant genealogical outcome (Avise & Ball 1991) could arise is if the extant population was derived from recent hybridization between Atlantic females and Gulf males followed mostly by unidirectional backcrossing to Gulf males. Clearly, information on nuclear genetic variation within and among recognized subspecies in the seaside sparrow assemblage will be needed to address this or other possibilities, and thereby to complete the phylogeographic picture.

As gauged by mtDNA (as well as historical geological evidence), the seaside sparrow assemblage appears to be subdivided into two major phylogeographic units. For management purposes, these phylogroups clearly should warrant recognition as 'evolutionarily significant units' (Ryder 1986; Dizon *et al*. 1992; Moritz 1994; Bowen 1998), especially if they prove to be divergent in nuclear genes also. Perhaps the current mtDNA findings on the Cape Sable seaside sparrow will rekindle interest in the taxonomic and management implications of phylogeographic data in the complex.

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Before his recent 'retirement', Bill Nelson for more than a decade was research coordinator of the Avise laboratory, which is devoted to studies in molecular ecology and conservation genetics. Tylan Dean has been involved in field related research on the Cape Sable seaside sparrow.
