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Initial Comparison of Genetic Population Structure of *Mustelus canis* using the mitochondrial gene, NADH-2

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Introduction:

Mustelus canis, the dusky smoothhound shark, is a widely distributed, coastal species that is caught in waters from Canada through Florida, throughout the Gulf of Mexico and into southern Argentina (Compagno 2005). This species supports commercial and recreational fisheries throughout its range and is one of the most abundant sharks in the U.S. Atlantic (NOAA 2010). Bigelow and Schroeder (1948) observed seasonal migrations of this species from the Carolinas through southern New England and hypothesized that there may be several distinct stocks of this species in U.S. waters. Here, we test the null hypothesis of genetic homogeneity by comparing sequences of the mitochondrial gene, NADH-2, from individuals caught in several localities in the Atlantic, as well as in the Gulf of Mexico.

Methods:

The population structure of the dusky smoothhound shark, *Mustelus canis,* was examined by direct sequencing of the 1047bp mitochondrially-encoded NADH-2 gene. Fin clips were collected from specimens in the Atlantic; Massachusetts (MA), Delaware Bay (DB), Virginia (VA), North Carolina (NC), South Carolina (SC), Georgia (GA)) and from the Gulf of Mexico; Florida in deepwater (FL_Deep; sets above 208m from Deep-C), the western Gulf (GulfW: West of Mississippi River Delta, the eastern Gulf (GulfE; East of Mississippi River Delta). Whole genomic DNA was extracted using a modified Chelex resin extraction method (Estoup *et al*. 1996). The ND-2 gene was amplified using PCR primers specific for the 1047 bp ND-2 region of *M. canis:*

Forward Primer: 5'-CCA TAC CCC AAC CAT GTG GTT-3'

Reverse Primer: 5'-GCT TTG AAG GCT TTT GGT CTG-3'

Thirty microliter reactions containing 100 ng DNA, 1x PCR buffer, 0.5 U *Taq* DNA polymerase (GoTaq Flexi DNA Polymerase, Promega), 1.5 uM of each primer, 2.4 mM dNTPs, and 2.4 mM

$MgCl_2$. The PCR amplification profile was as follows; initial denaturation at 95°C for 3 min, 40 cycles of 95°C for 30 sec, 60°C for 1 min and 72°C for 1 min, and final extension of 72°C for 10 min. Amplicons were electrophoresed on 2.0% agarose gels, extracted and purified with a QIAquick Gel Extraction Kit (QIAGEN, www.qiagen.com). PCR products were sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) or Beckman Coulter (http:/beckmangenomics.com/genomic_services/sanger_dna_sequencing.html) for sequencing. Sequence chromatograms were corrected by eye and aligned using SEQUENCHER v.4.8 (Gene Codes Corp.). Unique haplotypes were identified using DNASP (Rozas et al. 2003).

Genetic homogeneity of mtDNA variation among locations was tested using a hierarchical AMOVA, as implemented in ARLEQUIN; variance components that were assessed include differences among groups (Atlantic versus Gulf), among samples within groups, and among samples. Pairwise F_{ST} values between sampling localities were estimated using ARLEQUIN v. 3.5.1.3 (Excoffier et al. 1992; Schneider et al. 2000).

Results and Discussion:

One hundred and seventy one samples were successfully sequenced (1047 bp ND-2); there were 19 total haplotypes. One hundred seventeen individuals shared the central haplotype and there were 18 satellite haplotypes, most of which differed from the central haplotype by a single base-pair, two of which differed from the central haplotype by two-base-pairs. Of the satellite haplotypes, five were found solely in the Gulf of Mexico and 12 were found solely along the Atlantic coast.

The AMOVA results show that 4.31% of the total genetic variation was partitioned between the Atlantic and Gulf; results were significant (P = 0.028). Pairwise tests of F_{ST} also were significant in eight of twelve comparisons between the Gulf and the Atlantic (see Table 1).

This preliminary analysis of genetic variance among sample localities indicates that individuals of *Mustelus canis* in the Atlantic may be isolated from those that are found in the Gulf of Mexico, but it is important to note that these initial results only include comparisons of a single locus. A more comprehensive comparison of genetic variability among geographic localities utilizing nuclear-encoded microsatellite is in progress.

	DBVA	NC	SCGA	MA	FL_Deep	GulfW	GulfE
DBVA	----	**0.031**	0.011	0.012	**0.056**	0.029	**0.066**
NC	**0.045**	----	--0.019	--0.008	**0.052**	0.014	**0.046**
SCGA	0.225	0.946	----	-0.022	**0.042**	-0.003	**0.043**
MA	0.198	0.685	0.937	----	**0.042**	--0.007	**0.038**
FL_Deep	**0.045**	**0.000**	**0.018**	**0.009**	----	0.008	-0.033
GulfW	0.108	0.423	0.496	0.928	0.279	----	0.015
GulfE	**0.000**	**0.018**	**0.000**	**0.018**	0.829	0.378	----

Table 1: Pair-wise F_{ST} and F_{ST} *P*-values between localities: above diagonal: population pairwise F_{ST} values; below diagonal: F_{ST} *P*-value

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