

POPULATION GENETICS OF ATLANTIC STURGEON *ACIPENSER OXYRINCHUS*
BASED ON ANALYSIS OF MITOCHONDRIAL DNA

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Introduction

In what may be the largest genetic study of any acipenseriform, we surveyed mitochondrial DNA (mtDNA) variation in more than 600 specimens of *Acipenser oxyrinchus* (Atlantic sturgeon). Problems investigated included the relationships between the two forms that occur on the Atlantic and Gulf of Mexico coasts and also between *A. oxyrinchus* and *A. sturio* (European sea sturgeon), the stock structure of populations along the Atlantic and Gulf of Mexico coasts, the stock composition of the New York Bight commercial fishery, and gene flow among Gulf of Mexico stocks.

Taxonomic Investigations

In the U.S., the taxonomic status of threatened or endangered populations of vertebrates is important in determining the degree of protection they may receive under the Endangered Species Act (O'Brien & Mayr 1991). For *Acipenser oxyrinchus*, the Atlantic (*A. o. oxyrinchus*) and Gulf forms (*A. o. desotoi*) were designated subspecies based on differentiation of morphological features, although only two Gulf specimens were examined (Vladykov 1955). Wooley (1985) reanalyzed these morphological features of a larger sample of Gulf sturgeon and concluded that only one, relative spleen length, was diagnostic. However, values for relative spleen length of the two forms were not discrete and it may be that differences in these values are ecophenotypic in nature. We used direct sequencing of a hypervariable area (203 base pairs) of the control region of mtDNA to quantify the extent of genetic differentiation between the two putative subspecies (Ong et al. 1996). Representatives of each subspecies from populations across their distributions were surveyed and three fixed differences were found among 15 polymorphic sites. An additional two nucleotides were nearly fixed. Although polymorphisms also were detected within populations across the distribution of each subspecies, no fixed differences among populations were found. Ong et al. (1996) concluded that these data strongly supported the designation of subspecies of *A. oxyrinchus*.

The relationship between *A. oxyrinchus* and *A. sturio* has long been debated. These fish are found on opposite sides of the North Atlantic and are phenotypically very similar. *Acipenser oxyrinchus* occurs widely along the Atlantic coast of North America. *Acipenser sturio*, which

once had a similarly broad range in Europe and Asia, now occurs only in very low abundances in both the Gironde River, France, and the Black Sea (Rochard et al. 1990). Some workers have considered the Atlantic sturgeon to be synonymous with or a subspecies of *A. sturio* (Scott & Scott 1988; Birstein 1993), but Vladykov and Greeley (1963) and Magnin (1964) recommended they be given separate specific status, pending additional research. We obtained a tissue sample from one *A. sturio* specimen captured during 1994 in the Gironde River. We then compared a portion (203 bp) of the control region of mtDNA between *A. sturio* and *A. oxyrinchus*.

We found a minimum of 31, and a maximum of 33 nucleotide substitutions between the individual of *A. sturio* and more than 70 individuals of both subspecies of *A. oxyrinchus*. Three sites also exhibited nucleotide additions or deletions. In comparison, the number of nucleotide substitutions between any pair of specimens of the two subspecies of *A. oxyrinchus* ranged between five and eight, with no additions or deletions. Excluding additions or deletions, nucleotide divergence between *A. oxyrinchus* and *A. sturio* was approximately 15%, much higher than the maximum of 3.5% between the two subspecies of *A. oxyrinchus* (Ong et al. 1996). There are no unambiguous criteria for the interpretation of molecular data in determining taxonomic status (e.g., O'Brien & Mayr 1991, Wayne 1992). However, we believe that the level of differentiation observed argues strongly for full species status of each of the western and eastern Atlantic sea sturgeons.

Stock Structure Along the Gulf of Mexico

Populations of *A. oxyrinchus* occur in drainages of the Gulf of Mexico and along the Atlantic coast of North America. *Acipenser oxyrinchus desotoi* are considered threatened by U.S. Fish and Wildlife Service. Historically, *A. o. desotoi* were found in major river systems extending from central Florida to the Mississippi River; many of these drainages still host depleted populations (USFWS & GSMFC 1995). Efforts are being considered to restore depressed populations through hatchery supplementation. However, knowledge of the stock composition of *A. o. desotoi* was federally mandated prior to the initiation of restorative efforts, so that native gene pools are preserved. The Suwannee River population has been extensively studied over the past decade and probably contains the largest population of sturgeon along the Gulf of Mexico. Miracle & Campton (1995) examined the extent of genetic variation in sturgeon from the Suwannee River to determine if its population constituted a single homogeneous unit. Sequence analysis of 268 base pairs of a highly variable area of the mtDNA control region did not reveal significant genetic heterogeneity among sturgeon from different sampling locations.

We used restriction fragment length polymorphism (RFLP) and sequencing analyses of mtDNA to assess the stock structuring of *A. o. desotoi* populations among eight drainages extending from the Mississippi River to the Suwannee River (Stabile et al. 1996). RFLP analysis using four diagnostic restriction enzymes yielded eight composite haplotypes; genotypic diversity indices (Nei & Tajima 1981) ranged between 0.173 for the Choctawhatchee River sample to 0.732 for the Yellow River collection. Significant differences ($P < 0.05$) in haplotype frequencies indicated substantial geographic structuring of sturgeon populations; results from the RFLP ($N = 164$) and sequence ($N = 141$) analyses were largely congruent. Five regional or river-specific stocks were identified (from west to east): (1) Lake Ponchartrain and Pearl River, (2) Pascagoula River, (3) Escambia and Yellow rivers, (4) Choctawhatchee River, and (5) Apalachicola, Ochlockonee, and Suwannee rivers. These results suggest strong reproductive isolation of *A. o. desotoi* stocks on at least a regional basis, and point to the inadvisability of mixing of hatchery-reared progeny of broodstock from different Gulf rivers.

Stock Structure Along the Atlantic Coast

We used RFLP analysis of mtDNA with five diagnostic restriction enzymes to characterize the stock structure of populations of *A. o. oxyrinchus* along the Atlantic coast, including the St. Lawrence River, Quebec; St. John River, New Brunswick; Hudson River, New York; Edisto River, South Carolina; and four rivers in Georgia; the Altamaha, Ogeechee, Savannah, and Satilla (Waldman et al. 1996 a,b). Chi-square analysis showed the eight populations could be grouped as three highly differentiated ($P < 0.0001$) stocks: (1) Canadian (St. Lawrence and St. John rivers); (2) Hudson River; and (3) southeastern (Edisto, Savannah, Ogeechee, Altamaha, and Satilla). Composite haplotypes showed a clear cline in genotypic diversity indices (Nei & Tajima 1981) among populations that ranged from complete monomorphism (0.0) of the two Canadian populations to considerable polymorphism among southeastern populations (e.g., Edisto River: 0.646; Ogeechee River: 0.750). A latitudinal cline in genotypic diversity along the Atlantic coast is consistent with founder effects among northern populations that recolonized glaciated drainages from more genotypically diverse populations in southern, nonglaciated regions.

Mixed-Stock Analysis of the New York Bight Fishery

For some wide ranging species, fisheries have developed distant from spawning and nursery areas, and these fisheries may harvest individuals from more than one stock. For management purposes, it is important to quantitatively estimate the relative contributions of individual stocks to mixed fisheries to allow managers to protect threatened stocks at sites distant from their natal rivers. Successful application of genetic approaches to mixed-stock analysis is dependent on the existence of significant differentiation of genetic characters among spawning stocks which contribute to the mixed fishery (Utter & Ryman 1993, Xu et al. 1994). To conduct mixed stock analysis, frequencies of genotypes must be characterized in reference spawning stocks and in the mixed fishery.

A targeted coastal fishery for *A. o. oxyrinchus* has developed in recent years along the mid-Atlantic coast of New Jersey and New York (New York Bight). Waldman et al. (1996a) performed RFLP analysis of specimens of *A. o. oxyrinchus* from eight populations from Canada to Georgia and concluded based on haplotype frequency differences that three statistically discrete ($P < 0.0001$) stocks exist: (1) Canadian, (2) Hudson River, and (3) southeastern. Haplotypic frequency data of these stocks was then used in a mixture model (constrained least squares; Xu et al. 1994) to estimate the relative contributions of each of these stocks to a sample of Atlantic sturgeon ($N = 112$) from the fishery in the New York Bight off New Jersey. This analysis showed a 97% to 99% contribution from the Hudson River stock, with the remainder from the southeastern stock. The overwhelming contribution of the Hudson River stock was attributed both to (1) a hypothesized tendency for marine migrating Atlantic sturgeon to remain within the geographic provinces of their natal rivers (the Hudson River is within the Virginian province), and (2) to the absence of other robust Atlantic sturgeon populations within the Virginian province.

Gene Flow and Homing Fidelity

Most populations of sturgeons are anadromous or potamodromous and thus, migrate from marine or lake waters to rivers to spawn. However, almost nothing is known of the degree of homing fidelity shown by acipenseriforms. Although homing fidelity of fishes may be studied directly by means of mark-recapture (e.g., Melvin et al. 1986), the relative scarcity and high value of sturgeons precludes such an approach. An alternative is to assess homing fidelity indirectly through genetic analysis (Tallman & Healey 1994).

Homing fidelity of sturgeons through genetic analyses would best be assessed among populations in rivers that drain to a common water body and that historically have a stable geographic history to avoid confoundment by founder effects as a consequence of recolonizations. Stabile et al. (1996) used both RFLP and sequencing analysis of mtDNA to estimate gene flow among five stocks of *A. o. desotoi* that occur in eight drainages that feed the Gulf of Mexico between Mississippi and Florida. The five stocks were defined based on χ^2 analyses ($P < 0.05$) of haplotype frequencies; some stocks were equivalent to single populations, whereas others were regional stocks made up of two or more populations. Pairwise gene flow estimates (N_m) between stocks were derived from F_{st} values (Wright 1943) obtained via AMOVA analysis (Excoffier et al. 1992).

Pairwise estimates of gene flow (Table 1) among the Gulf stocks based on sequencing analysis ranged from 0.15 between the western (Lake Ponchartrain and Pearl River) stock and the Escambia River-Yellow River stock, to 1.2 between the Escambia River-Yellow River stock and the eastern stock (Apalachicola, Ochlockonee, and Suwannee rivers). Gene flow estimates derived from RFLP analysis were even lower on average, and ranged from 0.09 between the western and Choctawhatchee River stocks to 0.66 between the western and Escambia River-Yellow River stock.

These gene flow values are very low in comparison with estimates for other anadromous fishes. Estimated annual straying rates among populations of Pacific salmon have ranged between about 1% and 27% (reviewed in Adkinson 1996). Laughlin & Turner (1996) used two statistical methods to estimate N_m of *Morone saxatilis* (striped bass) among three Virginia tributaries of Chesapeake Bay; the private allele approach of Barton & Slatkin (1986) yielded an estimate of $N_m = 14.2$, whereas the F_{st} approach yielded an estimate of $N_m = 2.7$. In a mark-recapture study, Melvin et al. (1986) estimated an annual straying rate of 3% among Canadian populations of *Alosa sapidissima* (American shad).

Moreover, the low gene flow estimates for *A. o. desotoi* were obtained across populations that occur in eight rivers, the mouths of which are arrayed across little more than 500 km of coastline. Sturgeon from these rivers have the opportunity to mix in the Gulf of Mexico during winter. These mtDNA data show that despite the geographic proximity of these rivers, stocks of *A. o. oxyrinchus* generally exchange less than one female per generation, a level sufficient to permit genetic differentiation at the stock level (Adkinson 1996). Gene flow estimates also were generally higher among proximal stocks, suggesting that what straying occurs does so in 'stepping stone' fashion (Kimura & Weiss 1964) in which migrants among semi-isolated populations are exchanged chiefly with neighboring populations. If this is true for *A. o. desotoi*, then such spatially restricted straying should have contributed to the geographic structuring observed among these populations (Adkinson 1996). Stabile et al. (submitted) hypothesized that the homing imperative of *A. o. desotoi* for spawning purposes is strong, but that it may be reinforced by metabolic constraints. *Acipenser oxyrinchus desotoi* return to rivers from the Gulf of Mexico to summer near cold water springs; tagging has shown that individuals are recaptured at the same cool water refuges in which they were first tagged (Clugston et al. 1995). Low gene flow estimates indicate that natural recolonization of populations of *A. oxyrinchus* would proceed slowly.

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