Progress Report for 2001

STOCK STRUCTURE AND GENETIC DIVERSITY IN THE ROBUST REDHORSE (MOXOSTOMA ROBUSTUM) FROM ATLANTIC SLOPE RIVERS

Continuing Tailored Collaboration Research Project

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Mitochondrial and nuclear DNA divergence in robust redhorse and silver redhorse from three populations

In this study, we compared the extent of genetic divergence between robust redhorse from the Oconee, Savannah, and Pee Dee rivers to that in silver redhorse from the same rivers. The overall objective was to evaluate the taxonomic importance of genetic differences that we previously reported in mitochondrial DNA (mtDNA) sequences among robust redhorse from two of these populations. Mitochondrial DNA control region sequence and microsatellite analyses of nuclear DNA (nDNA) were used to quantify genetic differences among these robust redhorse and silver redhorse populations.

All robust redhorse from the Oconee River (n = 47) exhibited two mtDNA haplotypes (A and B) that were absent in robust redhorse from the other two populations (Figure 1). Similarly, robust redhorse from the Savannah River (n = 27) exhibited three mtDNA haplotypes (C, D, and D') that were absent in robust redhorse from elsewhere. Finally, robust redhorse from the Pee Dee River exhibited mtDNA haplotypes (E and E') that were only seen in that river. Overall mtDNA sequence divergence between robust redhorse from the Oconee and Savannah rivers averaged about 1.0%. In comparison, the two robust redhorse collected from the Pee Dee River in the years 2000 and 2001 exhibited much more genetic divergence from robust redhorse from each of the other two rivers (approximately 3%).

Mitochondrial DNA control region sequence analysis was also conducted on silver redhorse from these same rivers. Congruent results were obtained to those for robust redhorse with several exceptions. Most, but not all fish (17/19), from the Savannah River exhibited mtDNA haplotypes that were absent in fish from the Oconee (n = 20) or Pee Dee (n = 12) rivers (Figure 2). However, the extent of mtDNA sequence divergence among silver redhorse from these rivers was not as pronounced as that seen with robust redhorse. Pee Dee River silver redhorse were once again most divergent, but the extent of genetic divergence between silver redhorse from the Pee Dee and other rivers was only 1.0% compared to 3% for robust redhorse.

Microsatellite analysis was conducted on the same robust redhorse and silver redhorse samples described above using five of the microsatellite primer sets that we isolated and characterized from a robust redhorse library. Fixed nDNA allelic differences were observed among robust redhorse from these three populations at a single locus (RR37) and highly significant ($P < 0.01$) allelic frequency differences were observed at 3/4 of the remaining loci. At the RR37 locus, no overlap in molecular size was observed among robust redhorse from the three populations highlighting the extent of microsatellite allelic divergence among populations from these three rivers. Applying microsatellite analysis at these same loci to silver redhorse revealed highly significant ($p < 0.001$) allelic frequency differences at five loci among all three populations–but an absence of fixed differences.

Thus, results using both approaches on silver redhorse revealed congruent results, highly significant, but not fixed differences in population comparisons. In summary, our results indicate that populations of both redhorse species in rivers in Atlantic coastal slope rivers are highly genetically distinct. However, the magnitude of differences among robust redhorse populations slightly exceeds that among silver redhorse populations from the same rivers

supporting the suggestion that individual robust redhorse populations may warrant additional protection as evolutionary significant units.

Development and use of a simple molecular approach to distinguish robust redhorse and silver redhorse larvae

Indices of abundances of larval stages of robust redhorse are needed to identify environmental factors, which determine early life stage success. Morphological and ecological characteristics that unequivocally distinguish larvae of redhorse species are currently lacking. Analysis of DNA permits investigations from even the smallest life stages of fish by using the polymerase chain reaction (PCR). PCR also permits the analysis of the large numbers of samples frequently needed to address questions of ecological concern. Mitochondrial DNA (mtDNA) offers the advantage, that because it is maternally inherited, all progeny of a single female share identical mtDNA haplotypes.

We screened our data set of mtDNA control region sequences of silver redhorse and robust redhorse for informative polymorphic sites, sites that exhibited fixed differences between the two species, but showed no variation within each species. Several polymorphic nucleotide sites were detected that exhibited fixed differences between DNA from a small number of adult silver and robust redhorse, that were monomorphic within each species, and that could be distinguished using restriction fragment length polymorphism analysis. Computer analysis of these mtDNA sequences indicated that two restriction enzymes, *Hinf* I and *Ssp* I, recognized two polymorphic nucleotide sites that could be used to distinguish the mtDNA of the two species. *Hinf* I digests the mtDNA control region of silver redhorse into two DNA fragments, but not that of robust redhorse (Figure 3). Conversely, *Ssp* I digests the mtDNA control region of robust redhorse into two fragments, but not that of silver redhorse (Figure 4). Thus, use of the two enzymes provides replicate analyses in the identification of redhorse species and confirms that all DNAs can be digested with a restriction enzyme.

We next empirically tested the efficacy of this assay in distinguishing mtDNA isolated from known adult robust and silver redhorse. The mtDNA control region of both species was PCR amplified with previously described redhorse primers (Wirgin et al. 2001) and digested individually with *Hinf* I and *Ssp* I. As expected, mtDNA isolated from 40 adult silver and robust redhorse, respectively, exhibited fixed differences between the two species at these two diagnostic restriction enzyme digestion sites. Thus, the use of this assay was validated on substantial numbers of known samples.

This assay was then applied to two groups of larvae provided by University of Georgia--Athens ($n = 56$ and $n = 10$). All larvae were unequivocally identified as silver redhorse. A third group of 53 unidentified larvae were provided by UGA in November 2001. Of these, mtDNA haplotypes with both restriction enzymes were obtained for 37. We were unable to obtain any mtDNA data for 13 fish, and for 3 fish mtDNA data was only obtained for one restriction enzyme. Of the 37 fish that were scored with both restriction enzymes, 34 were identified as robust redhorse and 3 as silver redhorse. In summary, we developed a simple molecular assay, which with 100% accuracy can be used routinely to distinguish large numbers of larvae from these two species.

Figure 1
Frequencies of mtDNA Control Region Haplotypes in Robust Redhorse from Three Populations

		MtDNA Haplotype						
River	N	A	B	C	D	D'	E	E'
Oconee	44	20	24	0	0	0	0	0
Ocmulgee	3	1	2	0	0	0	0	0
Savannah	27	0	0	13	13	1	0	0
Pee Dee	2	0	0	0	0	0	1	1

Figure 2
Frequencies of mtDNA Haplotypes in Silver Redhorse from Three Populations

Haplotypes

River	F	F'	G	H	H'	I	I'	J	J'	K
Oconee (20)	7	9	4							
Savannah (19)	2			9	1	6	1			
Pee Dee (12)								10	1	1

Figure 3

Hinf I digestion of the robust redhorse and silver redhorse mtDNA control region

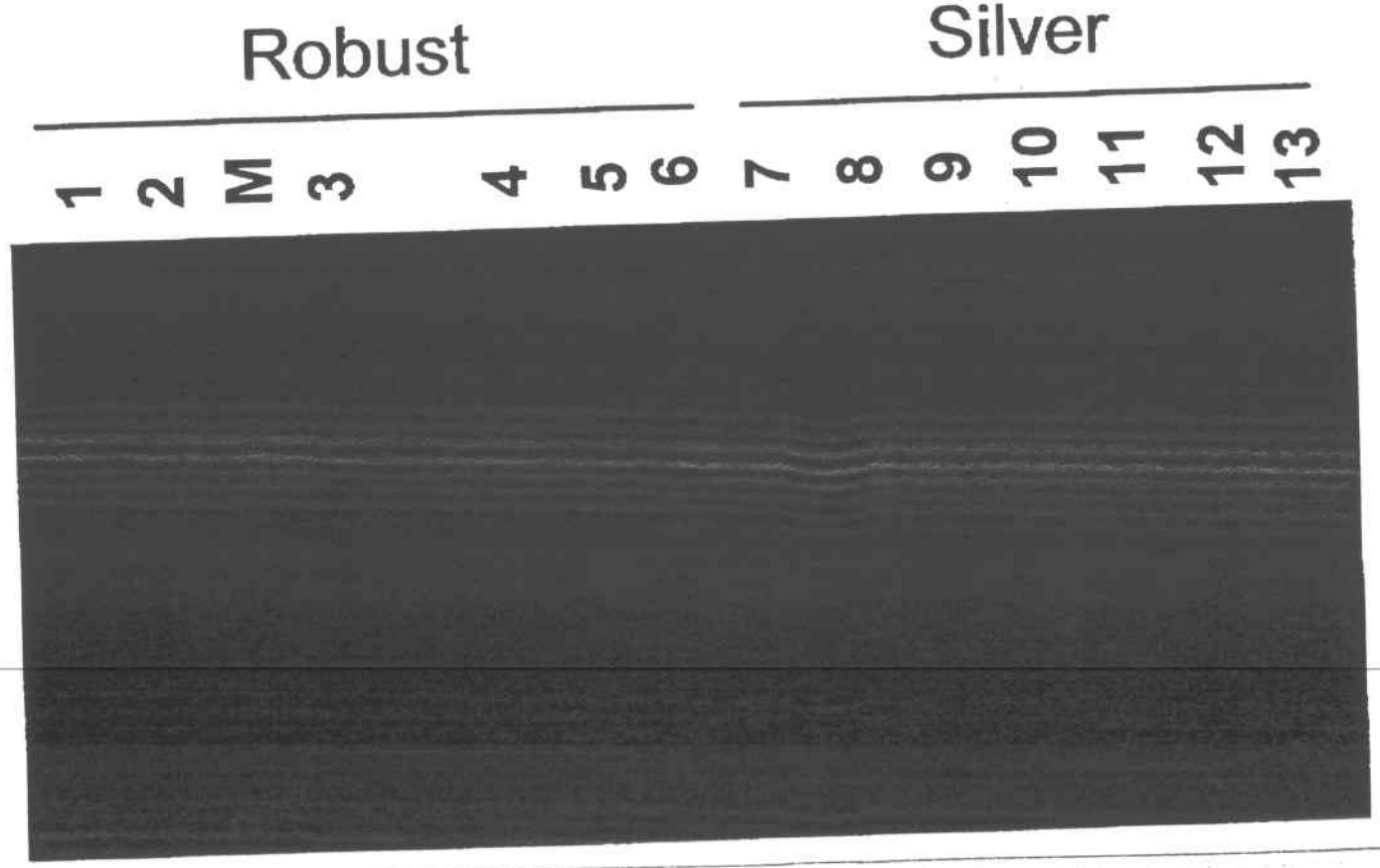

Figure 4
Ssp I digestion of the robust redhorse and silver redhorse mtDNA control region