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Genetic Identity of Pallid and Shovelnose Sturgeon (*Scaphirhynchus albus* and *S. platorynchus*)

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Pallid and shovelnose sturgeon are electrophoretically indistinguishable at 37 loci. They share the same allele at 34 monomorphic loci and have similar allelic frequencies at three polymorphic loci. This complete lack of genetic divergence between species is unusual among fish that have been studied. The close genetic similarity is apparently due to recent or incomplete reproductive isolation accompanied by rapid morphological differentiation.

THE taxonomic status of the pallid sturgeon (*Scaphirhynchus albus*) and the shovelnose sturgeon (*S. platorynchus*) has been debated since the beginning of this century. Forbes and Richardson (1905) put these fish into separate genera, *Parascaphirhynchus albus* and *Scaphirhynchus platorynchus*. Berg (1911, 1948) supported the placement of *albus* into the genus *Scaphirhyn-*

TABLE 1. FREQUENCIES OF THE COMMON ALLELE AT THREE POLYMORPHIC LOCI IN *Scaphirhynchus*.

Sample area	No. of fish	Gpi-1	Pgm-1	Pgm-2
S. platorynchus				
Missouri R., below Ft. Peck Reservoir, MT	35	0.871	0.971	1.000
Yellowstone R., at mouth of the Tongue R., MT	38	0.882	0.974	0.987
Missouri R., near Brownville, NE	25	0.962	0.960	0.980
Missouri R., near Kansas City, MO	9	0.889	0.944	1.000
Mississippi R., at mouth of the Chippewa R., WI	28	0.780	0.964	0.982
Mississippi R., near Cairo, IL	40	0.838	0.913	0.975
Pooled samples	175	0.866	0.954	0.986
S. albus				
Pooled samples	10	0.700	0.950	0.950
Hybrids				
Pooled samples	6	0.917	1.000	1.000

chus. Bailey and Cross (1954) presented convincing evidence of congeneric status for these sturgeons. They concluded that *S. albus* and *S. platorynchus* are readily separable and well-marked species using morphometric and meristic characteristics. They discounted the placement into separate genera, concluding that it would obscure their similarity in several fundamental features, i.e., the striking elongation and armature of the caudal peduncle as compared to other acipenseroids.

The pallid sturgeon is generally larger, much less abundant, and occurs only in the main stem of the Missouri and Mississippi rivers; the shovelnose sturgeon has a much larger range that extends into several major tributaries (Bailey and Cross, 1954). Live fish can usually be distinguished by color when first taken from the water (R. M. Bailey, pers. comm.). The primary morphological characteristics that separate these two species are head length, inner barbel length, the distance from the snout tip to the outer barbel and the height of the tenth lateral plate. Pallid sturgeon also have a larger number of dorsal and anal fin rays. Differences in structural features such as swim bladder size, belly plates and gill rakers are also apparent between these fish (Bailey and Cross, 1954). D. Carlson (pers. comm.) has found clear differences in food habits and modest differences in growth rate and habitat preference.

The present endangered status of pallid sturgeon has rekindled interest in these species. D. Carlson (pers. comm.) has recently found evidence of interbreeding between the pallid and shovelnose sturgeon; 15 sturgeon collected from the Mississippi and Missouri rivers appear to be hybrids based on morphological and ecological criteria. This observation has prompted two questions: 1) How genetically different are these two species? and 2) Do these species hybridize? We used electrophoretic analysis in an attempt to answer these questions.

METHODS

Shovelnose sturgeon were collected from the lower Tongue-Yellowstone River drainage and the Missouri River in Montana, the Mississippi and Missouri rivers in Missouri, and the Chippewa River in Wisconsin (Table 1). Pallid sturgeon and suspected hybrids were collected from several locations in the Mississippi and Missouri rivers from Missouri. These collections were pooled because of the small number of individuals sampled.

The following 27 enzymes and a general protein stain were screened for adequate enzyme activity and resolution in samples of muscle, liver, eye, heart, intestine and brain: aspartate aminotransferase, AAT, EC 2.6.1.1; alcohol dehydrogenase, ADH, EC 1.1.1.1; adenylate kinase, AK, EC 2.7.3.2; aldolase, ALD, EC 4.1.2.13; creatine kinase, CK, EC 2.7.3.2.; diaphorase, DIA, EC 1.6.4.3; esterase, EST, EC 3.1.1.1; fumerate hydratase, FUM, EC 4.2.1.2; glyceraldehyde phosphate dehydrogenase, GAP, EC 1.2.1.12; glutamate dehydrogenase, GDH, EC 1.4.1.2; glycerol-3-phosphate dehydrogenase, G3P, EC 1.1.1.8; glucose-6-phosphate dehydrogenase, G6P, EC 1.1.1.49; glucose-phosphate isomerase, GPI, EC 5.3.1.9; B-glucoronidase, GUS, EC 3.2.1.31; hexokinase, HK, EC 2.7.1.1; isocitrate dehydrogenase, IDH, EC

1.1.1.42; leucine aminopeptidase, LAP, EC 3.4.11.1; lactate dehydrogenase, LDH, EC 1.1.1.27; malate dehydrogenase, MDH, EC 1.1.1.37; malic enzyme, ME, EC 1.1.1.40; mannosephosphate isomerase, MPI, EC 5.3.1.8; peptidase, PEP, EC 3.4.–.–; phosphogluconate dehydrogenase, PGD, EC 1.1.1.44; phosphoglucomutase, PGM, EC 2.7.5.1; sorbitol dehydrogenase, SDH, EC 1.1.1.14; superoxide dismutase, SOD, EC 1.15.1.1; xanthine dehydrogenase, XDH, EC 1.2.3.2.

Only those isozymes that followed a simple genetic pattern were used in the analysis (Utter et al., 1974). The lack of breeding data and the apparent duplicate nature of some of these enzyme loci complicate the interpretation of the isozyme patterns (Ohno et al., 1969; Slyn'ko, 1976). Our estimates of the number of loci coding for different enzymes are conservative in that they reflect the minimum number of loci involved. For example, a single invariant band found in all individuals examined might represent the products of any number of loci with the same common allele coding for the protein considered. Therefore, the exact number of loci involved cannot be determined. In such situations we have chosen to treat the protein as if encoded by only one locus.

Horizontal starch gel electrophoresis techniques followed those of May et al. (1979). Staining followed the methods of Allendorf et al. (1977). The nomenclature used to describe the gene loci and the allele variants encoding the enzymes surveyed follows the system proposed by Allendorf and Utter (1979).

Results

Pallid and shovelnose sturgeon are indistinguishable at all the loci examined. The sturgeon samples had no detectable enzyme activity for five enzymes: ADH, ALD, DIA, FUM and HK. Several putative loci were unscoreable due to poor resolution: Ak-1, Gap-1, Gdh-3, Ldh-1, Ldh-2, Me-2 and Pgm-3. The same variable isozyme patterns in both species were observed at four enzymes coded by an estimated eight loci: Aat-3,4; Ldh-3,4; Ldh-5,6; Mdh-2 and Sod-1; however, the genetic basis of this observed variation could not be adequately determined. The above loci are not included in the genetic analysis.

Our samples of both species are monomorphic for the same allele at 33 loci: Aat-1, Aat-2, Ak-2, Ak-3, Ck-1, Ck-2, Ck-3, Est-1, Est-2, Gap-2, Gap-3, Gdh-1, Gdh-2, Gpi-2, Gpi-3, Gus-1, G3p-1, G3p-2, G3p-3, G6pdh-1, G6pdh-2, Idh-1, Lap-1, Mdh-1, Me-1, Me-3, Mpi-1, Mpi-2, Pep-1, Pep-2, Pgd-1, Sdh-1 and Xdh-1. They are also invariant at four zones of general protein staining in muscle extracts. Three loci are genetically variable: Gpi-1, Pgm-1 and Pgm-2 (Table 1). These loci display isozyme patterns similar to those reported in other fish species and conform to a simple genetic model.

Individuals that were suspected to be hybrids could not be identified because no diagnostic alleles were detected between the two sturgeon species. There are no statistically significant allele frequency differences between these species at any of the variable loci. Genetic identity (I; Nei, 1972) between these species is 0.999. Average heterozygosity (H) is low (*S. platorynchus*, H = 0.010; *S. albus*, H = 0.017). This is due, in part, to the exclusion of the variable loci whose genetic basis is not clear, but it is similar to that reported in paddlefish (*Polyodon spathula*), another ancient fish lineage (Carlson et al., 1982).

The allele frequencies between sampling areas of shovelnose sturgeon were compared to each other in order to estimate the amount of genetic divergence between samples (Table 1). All of the samples have similar allelic frequencies at the three polymorphic loci; there are no statistically significant differences between sampling areas. There appears to have been enough movement of fish, at least historically, to homogenize the allelic frequencies throughout the range examined. The similar allele frequencies is indicative of substantial genetic exchange (Allendorf and Phelps, 1981).

Discussion

These results are in contrast to the usual close agreement of the taxonomic relationships indicated by electrophoresis to those previously indicated by traditional systematic criteria. That is, these sturgeon are morphologically distinct, but have no detectable genetic differences.

This electrophoretic similarity is not due to the small number of pallid sturgeon samples. Nei and Roychoudhury (1974) have emphasized that more information is gained in an electrophoretic study of species comparisons by increasing the number of loci sampled rather than by increasing the number of individuals. Gorman and Renzi (1979) found that genetic distance estimates between lizard species are hard-

ly affected by sample size and that a single individual may be used to represent a species for interspecific comparisons. However, it is best to collect enough individuals so that genetic variation within species can also be used in estimating the extent of genetic divergence. Nei (1978) has presented a further theoretical basis for Gorman and Renzi's (1979) observations.

Electrophoretic techniques are well recognized as a valuable tool for determining systematic relationships and the extent of divergence between taxa (Avise, 1974). The data are objective, have a simple genetic basis and allow us to estimate the amount of gene flow between two gene pools. When gene flow between two populations ceases, genetic differentiation accumulates through the process of mutation, genetic drift and natural selection. This genetic divergence can be detected through electrophoresis as differences in allelic composition and allelic frequencies.

The possibility of not detecting differences because of the small number of loci assayed compared to the entire genome, or because of the many genetic changes that can occur to a protein which are not electrophoretically detectable, may overestimate the genetic similarity between taxa. However, the same biases apply to all studies of genetic similarity based on electrophoretic data. The degree of genetic differentiation detected between the pallid and shovelnose sturgeon is typical for samples taken from a single population within a species.

There are at least three possible explanations of the genetic similarity of these sturgeon: 1) shovelnose and pallid sturgeon represent a morphological polymorphism within the same species; 2) assortative mating with selection acting against intermediate morphological forms; and 3) these two species have recently become isolated and have not yet accumulated any genetically detectable differences.

The sympatric existence of two morphological types (i.e., morphotypes) within an interbreeding population may give rise to taxonomic separation by traditional ichthyological systematics (Sage and Selander, 1975; Turner and Grosse, 1980; Kornfield and Smith, 1981). However, it is unlikely that these sturgeon taxa represent alternative morphological types segregating in a single interbreeding population. The morphological differences are manifold and are thus likely to be controlled by many separate loci. Mating between these taxa should produce many intermediate types.

The allelic frequency identity indicates recent genetic exchange between these taxa. There apparently has been enough genetic exchange to maintain identity at isozyme loci but not for morphological features. Assortative mating causing some degree of reproductive isolation may be reinforced by the morphological differences (such as size and color) and ecological preferences (Hagen, 1967). The distinctiveness of the morphological types may also be reinforced by natural selection against intermediate types.

Another possible explanation is that these two species have only recently become reproductively isolated. Selection for different morphological types could have resulted in rapid divergence of morphology before genetic differences accumulated at isozyme loci.

We are thus left with two possible explanations for the genetic similarity of pallid and shovelnose sturgeon: incomplete reproductive isolation and recently established reproductive isolation. These alternatives are similar and probably impossible to distinguish at present.

Studies of allozymic variation in fish populations have generally supported existing taxonomic relationships. There are, however, some notable exceptions. Shaklee and Tamaru (1981) have detected two previously undescribed "species" of bonefish (*Albula vulpes*) that are morphologically nearly identical and yet show allozymic divergence ($I = 0.313$) comparable to that found between species in different genera. In contrast, previously described separate species have been found to actually represent morphological or trophic types segregating within an interbreeding population of a single biological species (Sage and Selander, 1975; Turner and Grosse, 1980; Kornfield and Smith, 1981). Avise et al. (1975) have described allozymic divergence between two species of minnows (*Hesperoleucus symmetricus* and *Lavinia exilicauda*) that is comparable to what we have found between these sturgeon species. The two minnow species are indistinguishable at 23 out of 24 loci ($I = 0.948$) but are morphologically and ecologically distinct and exhibit strong prezygotic isolating barriers.

Traditional systematics and taxonomy are based on a comparison of phenotypes, with the underlying assumption that the phenotype is a reflection of the genotype. Modern molecular techniques allow direct comparison of the genotypes of organisms. The sometimes startling discordance between morphological and mo-

lecular comparison of taxa presents a new challenge. It is becoming increasingly clear that an examination of both the phenotypic and genotypic characteristics of organisms must be made to gain an understanding of their evolutionary relationships.

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Literature Cited

Allendorf, F. W., N. Mitchell, N. Ryman and G. Ståhl. 1977. Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. Hereditas 86:179–190.

———, and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Canad. J. Fish. Aquat. Sci. 38:1507–1514.

———, and F. M. Utter. 1979. Population genetics, p. 407–454. *In:* Fish physiology, 8. W. S. Hoar, D. J. Randall and J. R. Brett (eds.). Academic Press, New York.

Avise, J. C. 1974. Systematic value of electrophoretic data. Syst. Zool. 23:465–481.

———, J. J. Smith and F. J. Ayala. 1975. Adaptive differentiation with little genetic change between two native California minnows. Evolution 29:411–426.

Bailey, R. M., and F. B. Cross. 1954. River sturgeons of the American genus *Scaphirhynchus*: characters, distribution, and synonymy. Mich. Acad. Sci. Arts Let. 39:169–208.

Berg, L. S. 1911. Faune de la Russie et des pays limitrophes. Poissons (Marsipobranchii et Pisces), 1. St. Petersburg. (In Russian.)

Berg, L. S. 1948. Fresh water fishes of the U.S.S.R. and adjoining countries, 1. Academy of Science U.S.S.R., Moscow. (In Russian.)

Carlson, D. M., M. K. Kettler, S. E. Fisher and G. S. Whitt. 1982. Low genetic variability in paddlefish populations. Copeia 1982:721–725.

Forbes, S. A., and R. E. Richardson. 1905. On a new shovelnose sturgeon from the Mississippi River. Bull. Illinois State Lab. Nat. Hist. 7:37–44.

Gorman, G. C., and J. Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979: 242–249.

Kornfield, I. L., and D. Smith. 1981. Direct evidence of conspecificity in divergent cichlid fishes. Genetics 97:s59.

Hagen, D. W. 1967. Isolating mechanisms in threespine sticklebacks. J. Fish. Res. Board Canada 24: 1637–1692.

May, B., J. E. Wright and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. *Ibid.* 36: 1114–1128.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106:283–292.

———. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

———, and A. K. Roychoudhury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76:379–390.

Ohno, S., J. Muramota, C. Stenius, L. Christian, W. Kittrell and N. Atkin. 1969. Microchromosomes in holocephalian, chondrostean and holostean fishes. Chromosoma (Berl.) 26:35–40.

Sage, R. D., and R. K. Selander. 1975. Trophic radiation through polymorphism in cichlid fishes. Proc. Natl. Acad. Sci. USA 72:4669–4673.

Shaklee, J. B., and C. S. Tamaru. 1981. Biochemical and morphological evolution of Hawaiian bonefishes (*Albula*). Syst. Zool. 30:125–146.

Slyn'ko, V. I. 1976. Multiple molecular forms of malate dehydrogenase and lactate dehydrogenase in Russian sturgeon (*Acipenser güldenstäoti* Br.) and great sturgeon (*Huso huso* L.). Akademia Naukovi Biological Series Doklady 228:201–204.

Turner, B. J., and D. J. Grosse. 1980. Trophic differentiation in *Ilyodon*, a genus of stream-dwelling goodeid fishes: speciation versus ecological polymorphism. Evolution 34:259–270.

Utter, F. M., H. O. Hodgins and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations, p. 213–238. *In:* Biochemical and biophysical perspectives in marine biology. D. C. Malins and J. R. Sargent (eds.). Academic Press, New York.

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