Evidence for a Slowed Rate of Molecular Evolution in the Order Acipenseriformes

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A test of the hypothesis that the members of the order Acipenseriformes (sturgeons and paddlefishes) possess a slowed rate of molecular evolution was carried out by conducting relative-rate comparisons with representatives of four groups of teleost fishes (Cypriniformes, Elopomorpha, Salmonidae, and Percomorpha) using 21 nuclear or mitochondrial protein loci and the nuclear and mitochondrial small subunit rRNA genes, obtained from the literature or our own research. In 70 out of 81 comparisons between individual taxa (86%), acipenseriform sequences showed slower rates of change than the homologous teleost loci examined. When teleost sequences are considered together, 21 of the 23 loci show slower rates of substitution in the acipenseriform lineage. Teleost proteins show 1.85 times as many unique amino acid differences as acipenseriform proteins, when both are compared with outlier sequences. These results support a hypothesis of slowed molecular evolutionary rate in the Acipenseriformes.

Introduction

Sturgeon and paddlefish are considered ''living fossils'' (Gardiner 1984) because although the order Acipenseriformes first appeared in the fossil record approximately 200 MYA, it appears that they have not undergone much morphological change since the origin of the group. In addition, various karyotypic and genetic studies have found limited amounts of change in chromosomes and DNA sequences when different species of acipenseriforms are compared. For example, Kedrova, Wladytchenskaya, and Antonov (1980) found that almost all genome fractions of the four species of sturgeon they examined (*Huso huso*, *Acipenser ruthenus*, *A. stellatus*, and *A. gueldenstaedti*) were homologous. Birstein and Vasiliev (1987) concluded from their karyotypic study of three sturgeon species containing ~120 chromosomes (*H. huso*, *A. ruthenus*, and *A. stellatus*) and the results of other karyotypic studies of two species (*Polyodon spathula*—Dingerkus and Howell 1976 and *Scaphirhynchus platorynchus*—Ohno et al. 1969) containing ~120 chromosomes that the similarity of the karyotypes among these species indicates a slow rate of karyological evolution. In their study of six different species, De la Herrán et al. (2001) found low rates of mutation and homogenization for the *Hin*dIII satellite DNA family in sturgeon, which they note is not typical of most other satellite DNAs. Examination of partial and whole nuclear 18S rRNA genes in the Acipenseriformes by Birstein, Hanner, and DeSalle (1997) and J. Krieger and P. A. Fuerst (unpublished data) indicates a very small amount of difference in sequence between functional sequence variants of different species. The study by J. Krieger and P. A. Fuerst (unpublished data) detected only one base substitution in this gene between the functional (expressed) sequences from lake sturgeon (*A. fulvescens*) and North American paddlefish (*P. spathula*), which are members of two different families within the order. Examination of mitochondrial DNA (mtDNA) sequences, which are generally considered to be relatively quickly evolving in vertebrates, has also indicated low amounts of sequence divergence among different acipenseriform species relative to the age of the group as a whole (Brown et al. 1996; Birstein, Hanner, and DeSalle 1997; Birstein and DeSalle 1998; Krieger, Fuerst, and Cavender 2000). The small amount of divergence in various mtDNA sequences (D-loop and cytochrome *b*, 16S rRNA, 12S rRNA, cytochrome *c* oxidase subunit II, $tRNA_{Phe}$ and $tRNA_{Asp}$ genes) seen among species of this group could, in part, be because of relatively recent divergence times for the species in this group. The idea of recent divergence times was suggested by Choudhury and Dick (1998) in their study of the historical biogeography of sturgeon. The authors concluded: ''It appears that although the acipenserids are a geologically old group, the historical biogeography of surviving lineages is best explained by more recent geological and climatic changes.'' However, this probably does not completely explain the low levels of divergence among mtDNA sequences from acipenseriform species. Brown et al. (1996) carried out analyses of RFLP patterns and D-loop sequences in four species of North American sturgeon (*A. oxyrinchus*, *A. fulvescens*, *A. transmontanus*, and *A. medirostris*). They determined that to remain in accordance with the Miocene speciation events postulated to have separated eastern (*A. oxyrinchus* and *A. fulvescens*) and western (*A. transmontanus* and *A. medirostris*) sturgeon species in North America 7–12 MYA (Cavender 1986), the mutation rates of mtDNA in sturgeon must be two- to fourfold lower than that of mammalian mtDNA (Brown et al. 1996).

On the basis of the results of studies like these, it has been suggested (Birstein and Vasiliev 1987; Brown et al. 1996) that the members of the order Acipenseriformes possess reduced rates of evolution. Reduced evolutionary rates (as compared with mammals) have also been found in sharks and turtles, two groups which share some life history characteristics with sturgeon (long

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generation time, large body size, ectothermy, and low metabolic rate) (Avise et al. 1992; Martin, Naylor, and Palumbi 1992; Martin and Palumbi 1993; Martin 1999). In addition, reduced evolutionary rates were also discovered in bony fish (as compared with mammals) when the cytochrome *b* gene sequences of several species of Perciformes and Cypriniformes (Teleostei) were examined (Cantatore et al. 1994). The evidence presented above prompted us to formally test the hypothesis that the Acipenseriformes possess a reduced rate of molecular evolution. This study utilizes gene and protein sequences available from the literature and our research to carry out relative-rate tests comparing the rates of molecular evolution in the Acipenseriformes and another group of fish, the teleosts.

Materials and Methods

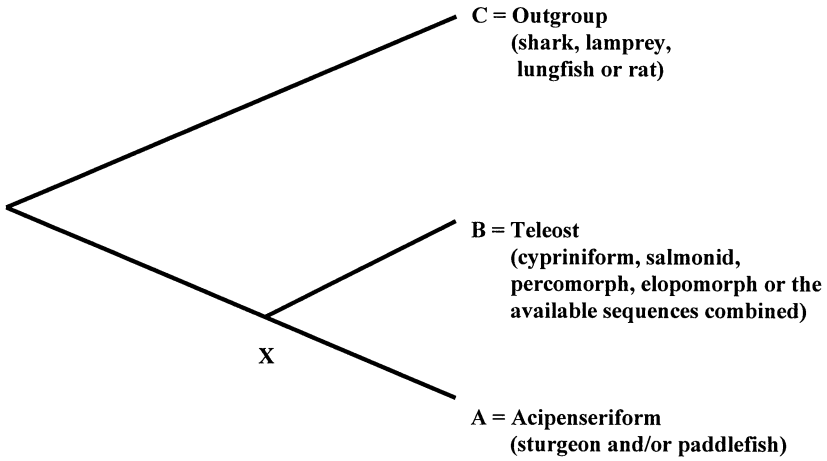
Gene and Protein Sequence Selection

The literature was searched in an attempt to identify genes or proteins for which an acipenseriform sequence, an outgroup (shark—Chondrichthyes, lungfish—Dipnoi, lamprey—Petromyzontiformes, or rat—Rodentia) sequence, and sequences from two or more of four teleost ingroups (one representative each from the Salmonidae, Cypriniformes, Percomorpha, and Elopomorpha) were available. Twenty-three genes or proteins were identified that fit these criteria, although sequences from all four teleost ingroups utilized in this study were not available for all the genes or proteins examined. Twenty of these sequences are nuclear: the 18S ribosomal RNA gene (18S rRNA gene), the beta-2 microglobulin protein, the CXC chemokine receptor 4 protein, the follicle-stimulating hormone protein, the glucagon protein, the glycoprotein hormone alpha subunit, the growth hormone protein, the heat shock protein 70, the insulin protein, the prolactin protein, the proopiomelanocortin protein, the proteolipid protein, the recombinase activating protein-1, the recombinase activating protein-2, the rhodopsin protein, the somatolactin protein, the thyroid-stimulating hormone protein, the triosephosphate isomerase protein, and the vitellogenin protein. The other three loci are mitochondrial: the 12S ribosomal RNA gene (12S rRNA gene), the cytochrome *c* oxidase subunit II protein, and the cytochrome *b* protein. The amino acid sequences of protein-coding genes were used instead of the nucleotide sequences because the distant relationships between the groups used in this study has likely resulted in the saturation of the nucleotide sequences with mutations, making them less informative for relative-rate analyses. The use of a closely related outgroup for relative-rate tests makes the statistical test of rate constancy more effective in rejecting the null hypothesis of equal rates of molecular change between the ingroup taxa. The outgroup most widely believed to be most closely related to acipenseriforms and teleosts is the order Polypteriformes. They have been suggested to be the sister group of Acipenseriformes + Neopterygii (gars, bowfin, and teleosts) by both morphological and molecular phylogenetic studies (Patterson 1982; Lauder and Liem 1983; Lê Lecointre, and Perasso 1993; Bemis, Findeis, and Grande 1997; Venkatesh, Ning, and Brenner 1999). Ideally, sequences from species belonging to the Polypteriformes should therefore be used to represent the outgroup. Unfortunately, relatively few polypteriform gene or protein sequences are available in the literature. Therefore, shark sequences were used when available, then lamprey, then lungfish, and if a fish outgroup sequence was not available, a mammal sequence (rat) was used. When there was more than one sequence reported from a species for a gene or protein in the literature, all available sequences were used together to represent that species. If both sturgeon and paddlefish sequences were available for a particular gene or protein, they were used together to represent the Acipenseriformes. This would compensate for possible rate differences within the Acipenseriformes when comparisons were made with the evolutionary rates in the teleosts (although, as discussed, there is little evidence for large amounts of DNA sequence divergence among acipenseriform species). One of the programs used for the analysis, PHYLTEST, allows multiple sequences to be used in representing a particular lineage (Takezaki, Razhetsky, and Nei 1995). A list of the species used in the relative-rate tests for each gene or protein, along with the GenBank accession numbers for their sequences, is given in the supplementary materials section.

Sequence Alignment and Relative-Rate Analyses

The 18S and 12S rRNA gene sequences were aligned by eye using the computer program ESEE (Cabot and Beckenbach 1989) with the aid of secondary structures created for the 18S and 12S rRNAs of *P. spathula* based on currently accepted models (Gutell 1994). Structural loop regions of the 18S rRNA gene sequences that could not be reliably aligned because of differences in length were omitted from the analyses. The 18S rRNA gene alignment was 1,787 base pairs in length, and the 12S rRNA gene alignment was 997 base pairs in length. The two rRNA gene alignments may be found in the alignment database at the European Bioinformatics Institute (http://www.ebi.ac.uk) (see *Supplementary Material* for a list of the accession numbers).

The cytochrome *b*, cytochrome *c* oxidase subunit II, glucagon, glycoprotein hormone alpha subunit, heat shock protein 70, insulin, recombinase activating protein-1, and recombinase activating protein-2 protein sequences were easily aligned by eye using the computer program ESEE (Cabot and Beckenbach 1989). The beta-2 microglobulin, CXC chemokine receptor 4, follicle-stimulating hormone, growth hormone, lutenizing hormone, prolactin, proopiomelanocortin, proteolipid protein, rhodopsin, somatolactin, thyroid-stimulating hormone, triosephosphate isomerase, and vitellogenin protein sequences were aligned using the computer sequence alignment program CLUSTALX (Thompson et al. 1997), and then any necessary adjustments to the alignments were made by hand. The insulin protein alignment includes sequences for both the A and B chains. The proopiomelanocortin protein alignment contains only regions of the molecule that represent corti-



Two-cluster test from Takezaki, Razhetsky and Nei (1995):

$L_{XA} = L_{AB} + L_{AC} - L_{BC}$

$L_{XB} = L_{AB} + L_{BC} - L_{AC}$

δ = difference between L_{XA} and $L_{XB} = L_{AC} - L_{BC}$*

*We expect $\delta = 0$ if rates of evolution are constant between the two lineages. If group A (Acipenseriformes) is evolving at a slower rate than group B (Teleostei- salmonids, cypriniforms, percomorphs and elopomorphs), then δ is negative. Alternatively, δ is positive if group A is evolving at a faster rate than group B.

FIG. 1.—Relative-rate test diagram and formulas illustrating the two-cluster test method (Takezaki, Razhetsky, and Nei 1995) used in the computer program PHYLTEST (Kumar 1996), which was used in this study.

cotropin, alpha-melanotropin, beta-melanotropin, and beta-endorphin, and any intervening regions that were similar in sequence, because these were the only regions that could be reliably identified and aligned for the seven species examined. A short sequence found at the amino end of the growth hormone of four species used in the tests was not available for the other two species used, so this region was omitted from the alignment. Only the amino half of the vitellogenin protein alignment was used for the tests because it was too difficult to accurately align the carboxyl end. Also, only partial follicle-stimulating hormone, lutenizing hormone, proteolipid protein, recombinase activating protein-1, recombinase activating protein-2, rhodopsin protein, and triosephosphate isomerase sequences were available, so just these available regions were used. (The recombinase activating protein-2 alignment is actually a combination of sequences from two different regions of the protein.) The lengths of the final protein sequence alignments were as follows: beta-2 microglobulin—122 amino acids, cytochrome *b*—382 amino acids, cytochrome *c* oxidase subunit II—230 amino acids, CXC chemokine receptor 4—360 amino acids, follicle-stimulating hormone—129 amino acids, glucagon—36 amino acids, glycoprotein hormone alpha subunit—120 amino acids, growth hormone—193 amino acids, heat shock protein 70—46 amino acids, insulin—53 amino acids, lutenizing hormone—144 amino acids, prolactin—226 amino acids, proopiomelanocortin—97 amino acids, proteolipid protein—153 amino acids, recombinase activating protein-1—326 amino acids, recombinase activating protein-2—259 amino acids, rhodopsin—288 amino acids, somatolactin protein—236 amino acids, thyroid-stimulating hormone—146 amino acids, triosephosphate isomerase—232 amino acids, and vitellogenin—1,102 amino acids. The alignments for these 21 protein sequences may also be found in the alignment database at the European Bioinformatics Institute (http://www.ebi.ac.uk) (see *Supplementary Material* for a list of the accession numbers).

The relative-rate test was carried out separately with representatives of all available teleost ingroups utilized in this study in order to determine if the trend in acipenseriform evolutionary rate was consistent when acipenseriforms were compared with different teleost species. In an attempt to compensate for possible rate differences among the teleost species, the rate test for each gene was also carried out using the available teleost sequences included in the study for that gene combined together as the ingroup, allowing the acipenseriform rate to be compared with the overall teleost rate. For each gene or protein examined, the computer program PHYLTEST (Kumar 1996) was used to carry out the two-cluster relative-rate test of Takezaki, Razhetsky, and Nei (1995), which is illustrated in figure 1. The test uses, as a reference taxon, an outgroup that diverged from the lineage leading to the two taxa being compared before the latter diverged from each other. The reference taxon

allows indirect comparison of the amount of change found in the lineages leading to each of the ingroups since the two ingroups diverged from one another (point X in fig. 1). This is possible because one can measure the amount of change that has occurred between the outgroup and each ingroup, as well as between the two ingroups by calculating genetic distances. The formulas shown in figure 1 illustrate how these genetic distances can be used to determine the amount of change taking place in the lineages leading to each ingroup since they diverged from one another (AX and BX in fig. 1). In this way, one can obtain a comparison of the relative rates of evolution in the two ingroups without knowing the exact timing of their divergence from the fossil record. For relative-rate comparisons of protein sequences, the Poisson correction distance was used, whereas the Kimura two-parameter distance (Kimura 1980) was used for tests involving gene sequences. These corrected distances were chosen because of the distant evolutionary relationships between the taxa compared in the tests. PHYLTEST (Kumar 1996) was also used to conduct a two-tailed normal deviate test (Takezaki, Razhetsky, and Nei 1995) for each relative-rate comparison to determine if the differences in evolutionary rates between the acipenseriforms and teleosts were statistically different form zero at the 5% level.

We have also utilized the nonparametric relative-rate test of Tajima (1993) to assess the significance of sequence differences in a three taxa situation. This avoids statistical complications that can arise when assuming specific patterns of amino acid or nucleotide substitution. Calculation of relative-rate tests with Tajima's nonparametric relative-rate test was performed using MEGA Version 2.1 (Kumar et al. 2001). Levels of significance observed in the nonparametric test were compared with those obtained using PHYLTEST. Further, the nonparametric test allows the identification of the number of unique amino acid changes that have occurred in each of the derived lineages (i.e., acipenseriform or teleost) and allows a quantification of the deficiency (or excess) of amino acid changes in the acipenseriform lineage relative to the teleost lineage.

Because our conclusions are not necessarily based on the significance of any single locus comparison, we have also considered the overall patterns of rates using a nonparametric sign test based on the binomial distribution. Signs of the relative rate in the acipenseriform lineage compared with a specific teleost taxon lineage were examined, and the significance of the overall patterns of rate differences was determined. In addition, using the information obtained from the Tajima test on the number of unique changes, the proportion of unique changes at a locus in paired lineages was examined in the entire protein data set using paired *t*-tests.

Results

Table 1 shows a summary of the results of the two-cluster relative-rate tests of Takezaki, Razhetsky, and Nei (1995) comparing acipenseriform sequences with those of a cypriniform, a salmonid, a percomorph, an elopomorph, or all available teleost groups combined. Seventy of 81 comparisons (86%) between individual taxonomic groups showed the acipenseriform sequences evolving more slowly than those of the teleosts. When the four teleost groups are combined for analysis, 21 of the 23 loci show acipenseriform sequences evolving more slowly. The consistency of the results when using different outgroups to compare different gene-protein sequences indicates that the use of different taxa to represent the outgroup apparently did not affect the results. When different outgroup sequences were available for a locus, no difference was seen in the patterns of lineage differences or in the significance of the results. The results of 39 of the 104 separate locus comparisons were statistically significant at the 5% level by the two-tailed normal deviate test conducted in PHYLTEST (Kumar 1996), as indicated by asterisks in Table 1. Results from Tajima's test agree closely. The sign of all tests is seen to be the same as determined by PHYLTEST. The significance levels are very similar as well. Two comparisons that are significant in table 1 did not show significance using Tajima's test (beta-2 microglobulin and somatolactin, both involving percomorph comparisons), whereas four that are not significant in table 1 were found to show significantly slower changes in acipenseriforms using Tajima's test (insulin comparing percomorphs and elopomorphs, rhodopsin comparing cypriniforms, and cytochrome *c* oxidase subunit II involving percomorphs). For both tests, all the statistically significant comparisons showed the acipenseriform sequences to be evolving more slowly than the sequences of teleosts. Only one gene and four protein sequences were, in some cases, found to be evolving more quickly in acipenseriforms than in teleosts. Only two loci show a consistent pattern of more rapid evolution in the acipenseriform taxa. Glucagon was evolving more quickly in the acipenseriform in all five comparisons with teleosts, and acipenseriform lutenizing hormone was evolving more quickly in all comparisons, except in that with the percomorph.

When considering the overall pattern of relative rates, using the sign test on the direction of change noted in table 1, results showed a significant pattern of slower change when acipenseriform taxa were compared with each of the four teleost taxon groups ($P < 0.01$ for comparisons with cypriniform, salmonid, or percomorph taxa, and $P < 0.05$ when compared with Elopomorpha).

The relative proportion of unique amino acid differences in the data was evaluated (as identified from the alignments using the Tajima's relative-rate test in MEGA). In a comparison of outliers with cypriniform and acipenseriform sequences, 4,463 amino acid sites were aligned. Of these, 517 sites (11.6%) showed cyprinform-specific amino acids, whereas only 290 (6.5%) showed acipenseriform-specific changes. Using a paired *t*-test to evaluate differences over all protein loci, acipenseriform taxa showed a highly significant deficiency of changes ($t = 2.71$, df $= 19$, $P < 0.01$ for a one-tailed test). For the salmonid-acipenseriform comparison, 4,431 amino acid sites were compared, with 526 (11.9%) salmonid and 278 (6.3%) acipenseriform-spe-

Table 1
Results of Two-Cluster Relative-Rate Tests (Takezaki, Razhetsky, and Nei 1995) Carried Out with the Computer Program PHYLTEST (Kumar 1996) for Acipenseriforms and Teleosts

Gene or Protein Name	Teleost Ingroup				
	Cyprini-form	Salmonid	Perco-morph	Elopo-morph	All Available Ingroups Combined
Nuclear					
18S rRNA gene (partial)	—*[a]	—	—	+	—*
Beta-2 microglobulin	—	+	—*	NA[b]	—
CXC chemokine receptor 4	—	—	—	NA	—
Follicle-stimulating hormone (partial)	—	—*	—*	—	—*
Glucagon	+	+	+	+	+
Glycoprotein hormone alpha subunit	—	—	—*	—	—*
Growth hormone (partial)	—*	—*	—*	—*	—*
Heat shock protein 70	0[c]	—	—	NA	—
Insulin (A & B chains)	—	—	—	—	—
Lutenizing hormone (partial)	+	+	—	+	+
Prolactin	—	—	—	—	—
Proopiomelanocortin (partial)	—	—*	—*	—	—*
Proteolipid protein (partial)	NA	—*	—	NA	—
Recombinase activating protein-1 (partial)	—	—*	—*	NA	—
Recombinase activating protein-2 (partial)	—	—	—	NA	—
Rhodopsin (partial)	—	—*	—*	—	—*
Somatolactin	—*	—	—*	—*	—*
Thyroid-stimulating hormone	—*	—*	NA	—*	—*
Triosphosphate isomerase (partial)	—	NA	—	NA	—
Vitellogenin (partial)	—*	—*	—*	NA	—*
Mitochondrial					
12S rRNA gene	—	—	—*	—*	—*
Cytochrome *b*	+	—	—	—	—
Cytochrome *c* oxidase subunit II	—	—	—*	—	—*

[a] The symbol — indicates that the acipenseriform sequence(s) exhibited a slower rate of evolution than that of the teleost ingroup, whereas a + indicates that the acipenseriform sequence exhibited a faster rate of evolution. An asterisk next to the symbol indicates that the difference in evolutionary rates for that comparison was determined to be statistically significant at the 5% level by a two-tailed normal deviate test conducted in PHYLTEST.

[b] NA indicates that a sequence from the indicated ingroup was not available for comparison.

[c] 0 indicates that rates were identical for both groups.

cific changes. Differences over all protein loci evaluated by the paired *t*-test show acipenseriform taxa with a highly significant deficiency of changes ($t = 3.30$, df = 19, $P < 0.01$ for a one-tailed test). For the percomorph-acipenseriform comparison, 4,502 amino acid sites were compared, with 550 (12.2%) percomorph and 289 (6.4%) acipenseriform-specific changes. Differences over all protein loci in the paired *t*-test show acipenseriform taxa with a highly significant deficiency of changes ($t = 3.80$, df = 19, $P < 0.01$ for a one-tailed test). Finally, for the elopomorph-acipenseriform comparison, 2,151 amino acid sites were compared, with 208 (9.7%) elopomorph and 115 (5.3%) acipenseriform-specific changes. Differences over all protein loci in the paired *t*-test show acipenseriform taxa with a significant deficiency of changes ($t = 1.80$, df = 12, $P < 0.05$ for a one-tailed test). Overall, the proportion of unique changes in the four teleost lineages averaged 1.85 times that seen in the acipenseriform lineage.

Discussion

Our results have shown a significantly slower rate of molecular change in the lineage that gave rise to the modern sturgeon and paddlefish, compared with the primary fish lineage which gave rise to the teleost fishes. This is particularly interesting because a previous study that examined perciform and cypriniform cytochrome *b* gene sequences determined that teleosts have a slower rate of molecular evolution than mammals (Cantatore et al. 1994). Our results indicate that the molecular evolutionary rate in the Acipenseriformes is likely even further reduced than that in teleosts. About 86% of pairwise relative-rate comparisons between acipenseriforms and teleosts showed slower rates in the Acipenseriformes. An examination of the average pattern of amino acid substitution suggests that the rate of change may be almost twice as fast in the teleost line. In addition, the reduction in evolutionary rate seems to be common to both nuclear and mitochondrial sequences.

A number of hypotheses have been proposed to explain the apparent slowed rates of molecular evolution observed in other taxa. Among these, the hypotheses that seem to be most relevant to a consideration of acipenseriform-teleost contrasts involve the inverse relationships between body size, generation time, or meta-

bolic rate (or all) and rates of molecular evolution. The generation time hypothesis (Kohne 1970; Li, Tanimura, and Sharp 1987; Ohta 1993; Mooers and Harvey 1994; Li et al. 1996) is based on the assumption that species with shorter generation times (rapid sexual maturation) possess germ lines that have a larger number of DNA replication events per year, so that they have a greater chance of replication error per unit time. However, germ line DNA replication events may not always be directly correlated to generation time; species may differ in the number of cell divisions per generation (Chang et al. 1994; Li et al. 1996). The metabolic rate hypothesis (Martin and Palumbi 1993; Martin 1999) proposes that higher metabolic rates produce more DNA-damaging chemicals (particularly free oxygen radicals), which increases the mutation rate relative to species with lower metabolic rates.

Contemporary acipenseriform species possess long generation times and low metabolic rates. Although there are ranges in body size and ages at sexual maturity depending on the species considered, sturgeon and paddlefish are some of the largest freshwater fish species. In nature, these fish take a relatively long time to reach sexual maturity, as compared with other fish species (approximately 5–23 years depending on sex because females generally take longer than males) (Birstein 1993 and references therein). In addition, the large body size and ectothermy of these fish suggests that they possess a relatively low metabolic rate, which has also been supported by experimental evidence. Singer, Mahadevappa, and Ballantyne (1990) noted that the rate of oxygen use in a salmonid, as determined by Brett (1972), was 5.5 times higher than that in a white sturgeon (*A. transmontanus*) of similar weight, as determined by Burggren (1978). In a study of the metabolism of lake sturgeon, (*A. fulvescens*), Singer, Mahadevappa, and Ballantyne (1990) found that the levels of citrate synthase (an enzyme involved in the Krebs cycle and characteristic of oxidative metabolism) in the sturgeon heart were four times lower than those found in the hearts of salmonids (Ewart and Driedzic 1987), again suggesting an overall lower metabolic rate. Burggren, Dunn, and Barnard (1979) determined the weight-specific gill area of lamellar blood channels in white sturgeon to be among the lowest found in many fish species examined, which is believed to reflect a low activity level and metabolic rate in sturgeon. Additional studies (Burggren 1978; Burggren and Randall 1978) found that sturgeons have a relatively low resting metabolic rate that does not increase much (2–3 times) during maximum swimming speeds and is lower than that of many fish of comparable sizes tested at similar temperatures. The low metabolic rate of sturgeons is probably a consequence of their slow moving, bottom feeding lifestyle (Burggren, Dunn, and Barnard 1979; Singer, Mahadevappa, and Ballantyne 1990).

Thus, the long generation time and low metabolic rate (or both) of the Acipenseriformes could be responsible for the slowed rate of molecular evolution observed in sturgeon and paddlefish (see Brown et al. 1996; Birstein, Hanner, and DeSalle 1997; Birstein and DeSalle 1998; Krieger, Fuerst, and Cavender 2000). It should be noted, however, that some studies on mammalian taxa failed to find correlations between generation time, body size, or metabolic rate (or all) and evolutionary rate (Sarich and Wilson 1973; Bromham, Rambaut, and Harvey 1996; Gissi et al. 2000). Therefore, different factors may be influencing rates of molecular evolution in different groups, possibly even factors that have not yet been considered.

In conclusion, evidence has been presented for a reduced rate of molecular evolution in a sample of 23 nuclear and mitochondrial loci in the Acipenseriformes compared with teleost fishes by conducting relative-rate tests. This phenomenon may be related to the life history and metabolic characters possessed by sturgeon and paddlefish. A slowed molecular evolutionary rate in the Acipenseriformes would explain the observed low levels of genetic divergence observed among species of the group (for example: Brown et al. 1996; Birstein, Hanner, and DeSalle 1997; Birstein and DeSalle 1998; Krieger, Fuerst, and Cavender 2000) and may also help explain the recent discovery of the existence of intraindividual variation of the 18S rRNA gene in sturgeon (J. Krieger and P. A. Fuerst, unpublished data).

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DAN GRAUR, reviewing editor

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Errata

Evidence for a Slowed Rate of Molecular Evolution in the Order Acipenseriformes

Jeannette Krieger and Paul A. Fuerst
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Supplementary Material for this article is available at the MBE website: www.molbiolevol.org.

The Role of Nuclear Genes in Intraspecific Evolutionary Inference: Genealogy of the *transferrin* Gene in the Brown Trout

Agostinho Antunes, Alan R. Templeton, René Guyomard, and Paulo Alexandrino
Mol Biol Evol 19(8): 1272-1287. 2002

Materials and Methods, first paragraph, first sentence: citations were omitted. Sentence should read:

> The current geographic distribution of *TF* electromorphs was assessed by combining previously published data (Krieg and Guyomard 1985; Presa et al. 1994; Berrebi 1995; Largiadèr 1995; Largiadèr and Scholl 1995; Giuffra, Guyomard, and Forneris 1996; Largiadèr and Scholl 1996*b*; Largiadèr, Scholl, and Guyomard 1996; Antunes, Alexandrino, and Ferrand 1999; Berrebi et al. 2000) with new electrophoretic screenings from several geographic areas (31 European populations corresponding to 661 individuals; see *Supplementary Material* at MBE website: www.molbiolevol.org).

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SUPPLEMENTARY MATERIAL

List of GenBank accession numbers for gene and protein sequences used in the acipenseriform relative-rate study. Species names with asterisks indicate those species used as representatives of the outgroup taxa for each gene or protein tested, and the sequences of species names marked with # symbols were determined in our laboratory.

Nuclear genes and proteins:

18S rRNA gene (partial):	*Polyodon spathula*#	AF188371
	Acipenser fulvescens#	AF188389
	*Squalus acanthias**	M91179
	Oncorhynchus masou	AF243427
	Cyprinus carpio	U87963 + AF133089
	Fundulus heteroclitus	M99180
	Ophichthus rex	X98843
Beta-2 microglobulin:	*Acipenser baeri* 1-5	CAB61322–CAB61326
	*Rattus norvegicus**	P07151
	Oncorhynchus mykiss	AAB04656
	Cyprinus carpio	Q03422
	Oryzias latipes	BAB60851
CXC chemokine receptor 4:	*Acipenser ruthenus*	CAB60252
	*Rattus norvegicus**	O08565
	Oncorhynchus mykiss	CAA04493
	Cyprinus carpio	BAA32797
	Takifugu rubripes	FT:T005471
Follicle-stimulating hormone (partial):	*Acipenser baeri*	CAB93504
	*Scyliorhinus canicula**	CAC43235
	Oncorhynchus keta	AAA49408
	Cyprinus carpio	BAA20080
	Morone saxatilis	I50993
	Anguilla japonica	BAA36546

Glucagon:	*Polyodon spathula* 1& 2	S44471, S44472
	*Scyliorhinus canicula**	AAB31092
	Oncorhynchus mykiss	AAC59669
	Carassius auratus	P79695
	Tilapia nilotica	P81026
	Anguilla anguilla	C60840
Glycoprotein hormone alpha subunit:	*Acipenser baeri*	CAC43060
	*Scyliorhinus canicula**	CAC43234
	Oncorhynchus mykiss	BAB17685
	Ctenopharyngodon idella	P30983
	Dicentrarchus labrax	AAK49431
	Anguilla anguilla	P27794
Growth hormone (partial):	*Acipenser gueldenstaedti*	S21750
	*Prionace glauca**	P34006
	Oncorhynchus keta	A23154
	Cyprinus carpio	CAA36228
	Tilapia mossambica	AAC77876
	Anguilla japonica	AAA48535
Heat shock protein 70:	*Acipenser baeri*	AAF66236
	*Rattus norvegicus**	AAA41354
	Oncorhynchus mykiss	BAB72233
	Danio rerio	AAF70445
	Oreochromis mossambicus	CAA04673
Insulin (A & B chains):	*Acipenser gueldenstaedti*	P81423
	Polyodon spathula 1 & 2	S44469, S44470
	*Squalus acanthias**	P12704
	Oncorhynchus keta	P04667
	Cyprinus carpio	P01335
	Tilapia nilotica	P81025
	Anguilla rostrata	A61125
Lutenizing hormone (partial):	*Acipenser baeri* 1 & 2	CAB93502, CAB93503
	*Scyliorhinus canicula**	CAC43236
	Oncorhynchus mykiss	BAB17687
	Cyprinus carpio	P01235
	Morone saxatilis	AAC38019
	Anguilla anguilla	AAL37629

Prolactin:	*Acipenser gueldentstaedti*	AAB28396
	*Protopterus aethiopicus**	P33091
	Oncorhynchus keta	226011
	Cyprinus carpio	P09585
	Tilapia mossambica	P09319
	Anguilla anguilla	P33096
Proopiomelanocortin (partial):	*Acipenser transmontanus*	BAA13682
	Polyodon spathula A & B	AAD41263, AAD41264
	*Squalus acanthias**	BAA32606
	Oncorhynchus keta	P10000
	Cyprinus carpio 1& 2	CAA74968, CAA74967
	Tilapia mossambica	AAD41261
	Anguilla rostrata	AAF22344
Proteolipid protein (partial):	*Acipenser sp.*	AAL12847
	Squalus acanthias α*	P36963
	Oncorhynchus mykiss	P78926
	Takifugu rubripes	AAL12848
Recombinase activating protein-1 (partial):	*Polyodon spathula*	AAL12859
	Acipenser sp.	AF369056
	*Carcharhinus leucas**	AAB17267
	Oncorhynchus mykiss	AAA80281
	Danio rerio	AAC60365
	Takifugu rubripes	AAD20561
Recombinase activating protein-2 (partial):	*Polyodon spathula*	AAL12877 + AAL12878
	Acipenser sp.	AAL12875 + AAL12876
	*Chiloscyllium punctatum**	AAL12884
	Oncorhynchus mykiss	AAB18138
	Danio rerio	AAC60366
	Takifugu rubripes	AAD20562

Rhodopsin (partial):	*Acipenser* sp.	AAD54572
	Polyodon spathula	AAL11508
	*Galeus melastomus**	CAA76798
	Salmo salar	AAF44620
	Carassius auratus	P32309
	Pomatoschistus minutus	P35403
	Anguilla anguilla 1& 2	AAA99297, AAA99200
Somatolactin:	*Acipenser transmontanus*	O93262
	*Protopterus annectens**	O73847
	Oncorhynchus keta	A23729
	Carassius auratus	JC5418
	Sparus aurata	AAA98734
	Anguilla anguilla	Q90216
Thyroid-stimulating hormone:	*Acipenser baeri*	CAB93505
	*Rattus norvegicus**	CAA25684
	Salmo salar	AAC77908
	Cyprinus carpio	BAA20082
	Anguilla anguilla	CAA51908
Triosephosphate isomerase (partial):	*Acipenser brevirostrum*	AAK85201
	*Lethenteron reissneri**	BAA88480
	Danio rerio A & B	AAK85203, AAK85202
	Xiphophorus maculatus	AAK85204
Vitellogenin (partial):	*Acipenser transmontanus*	Q90243
	*Ichthyomyzon unicuspis**	S28974
	Oncorhynchus mykiss	Q92093
	Pimephales promelas	AAD23878
	Oreochromis aureus	AAD48085

<u>Mitochondrial genes and proteins</u>:

12S rRNA gene:	*Acipenser fulvescens*[#]	AF125595
	Polyodon spathula[#]	AF125594
	*Squalus acanthias**	Y18134
	Salmo salar	AF133701
	Cyprinus carpio	NC_001606
	Pagrus major	AP002949
	Anguilla anguilla	AF266495

Cytochrome *b*:	*Scaphirhynchus suttkusi*	AAD11492
	Polyodon spathula	CAC19613
	*Squalus acanthias**	CAA77061
	Salmo salar	AAD04745
	Cyprinus carpio	CAA43342
	Oreochromis niloticus	BAA88867
	Anguilla rostrata	AAC98879
Cytochrome *c* oxidase subunit II:	*Acipenser fulvescens*[#]	AF125653
	Polyodon spathula[#]	AF125652
	*Squalus acanthias**	CAA77052
	Salmo salar	AAD04736
	Cyprinus carpio	CAA43340
	Dicentrarchus labrax	S45491
	Conger myriaster	BAB46995

The European Bioinformatics Institute alignment database accession numbers for gene and protein sequence alignments used in the acipenseriform relative-rate study gene are as follows: 18S rRNA gene--ALIGN_000269, Beta-2 microglobulin—ALIGN_000268, CXC chemokine receptor 4—ALIGN_000021, Follicle-stimulating hormone—ALIGN_000254 , Glucagon—ALIGN_000022, Glycoprotein hormone alpha subunit—ALIGN_000255, Growth hormone—ALIGN_000023, Heat shock protein 70—ALIGN_000256 , Insulin (A & B chains) —ALIGN_000257, Lutenizing hormone—ALIGN_000258 , Prolactin—ALIGN_000027, Proopiomelanocortin—ALIGN_000259, Proteolipid protein—ALIGN_000265, Recombinase activating protein-1—ALIGN_000270, Recombinase activating protein-2—ALIGN_000271, Rhodopsin—ALIGN_000272, Somatolactin—ALIGN_000274, Thyroid-stimulating hormone—ALIGN_000266, Triosephosphate isomerase—ALIGN_000273, Vitellogenin—ALIGN_000031, 12S rRNA gene—ALIGN_000264, Cytochrome *b*—ALIGN_000253 and Cytochrome *c* oxidase subunit II—ALIGN_000267.