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Phylogeny of the Acipenseriformes: cytogenetic and molecular approaches

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Synopsis

The review of the data on karyology and DNA content in Acipenseriformes shows that both extant families, the Polyodontidae and Acipenseridae, originated from a tetraploid ancestor which probably had a karyotype consisting of 120 macro- and microchromosomes and DNA content of about 3.2–3.8 pg per nucleus. The tetraploidization of the presumed 60-chromosome ancestor seems to have occurred at an early time of evolution of the group. The divergence of the Acipenseridae into Scaphirhyninae and Acipenserinae occurred without polyploidization. Within the genus *Acipenser*, polyploidization was one of the main genetic mechanisms of speciation by which 8n and 16n-ploid species were formed. Individual gene trees constructed for sequenced partial fragments of the 18S rRNA (230 base pairs, bp), 12S rRNA (185 bp), 16S rRNA (316 bp), and cytochrome *b* (270 bp) genes of two Eurasian (*A. baerii* and *A. ruthenus*) and two American (*A. transmontanus* and *A. medirostris*) species of *Acipenser*, *Huso dauricus*, *Pseudoscaphirhynchus kaufmanni*, *Scaphirhynchus albus*, and *Polyodon spathula* showed a low level of resolution; the analysis of a combined set of data for the four genes, however, gave better resolution. Our phylogeny based on molecular analysis had two major departures from existing morphological hypotheses: *Huso dauricus* is a sister-species to *Acipenser* instead of being basal to all acipenseriforms, and *Scaphirhynchus* and *Pseudoscaphirhynchus* do not form a monophyletic group. The phylogenetic tree constructed for the cytochrome *b* gene fragments (with inclusion of 7 additional species of *Acipenser*) supported the conclusion that octoploid species appeared at least three times within *Acipenser*.

Introduction

Although a few species of *Acipenser* require revision, usually 24–25 extant sturgeon and two paddlefish species, *Polyodon spathula* and *Psephurus gladius*, are included in the Acipenseriformes (Rochard et al. 1991, Birstein 1993a). The extant members of this order form the monophyletic sistergroup of all extant Neopterygii (e.g., Lepisosteidae, Amiidae, and Teleostei; Bemis et al. 1997 this volume). Most ichthyologists regard Polypteridae as the sister group of Acipenseriformes + Neopterygii (Patterson 1982). A comparison of partial sequences of 28S rRNAs supported this relationship (Le et al. 1993). In contrast to earlier works, Grande & Bemis (1991) concluded that paddlefishes and sturgeons are sister taxa, and that extinct Mesozoic genera such as *Chondrosteus* lie outside this clade.

Within Acipenseriformes, all workers agree that the Acipenseridae and Polyodontidae diverged prior the Late Cretaceous (Berg 1948a, Yakovlev 1977, 1986, Grande & Bemis 1991, Jin 1995, Grande & Bemis 1996, Bemis et al. 1997). Within the Acipenseridae, the subfamily Scaphirhynchinae (Central Asian species of *Pseudoscaphirhynchus* plus North American species of *Scaphirhynchus*) was traditionally considered the sister group of all other sturgeons (Berg 1905, Vasiliev 1985). Another interpretation, based on osteology, was proposed by Findeis (1993, 1997 this volume), who found that *Huso* lacks many characters found in *Acipenser*, *Scaphirhynchus* and *Pseudoscaphirhynchus*. On this basis, he considered Scaphirhynchinae as a derived group within Acipenseridae (Findeis 1997). This point of view was disputed since the description of the *Pseudoscaphirynchus* species 120 years ago (Kessler 1877, Berg 1905), while other researchers considered Scaphirhyninae as the oldest group within Acipenseridae (Bogdanov 1887). The examination of generic relationships within Acipenseriformes using molecular phylogenetic methods was one of the main goals of this paper.

According to Nesov & Kaznyshkin (1977), extant species of *Acipenser* belong to different evolutionary lineages which diverged long time ago. Artyukhin & Andronov (1990) and Artyukhin (1995) proposed that there were at least four main regions in which the speciation and spread of sturgeons took place: (1) the Ponto-Caspian area; (2) China-western America; (3) the Atlantic area; and (4) eastern North America. The group of Ponto-Caspian species includes most of the Eurasian species (*Acipenser gueldenstaedtii*, *A. persicus*, *A. stellatus*, *A. ruthenus*, *A. nudiventris*, *Huso huso*, *A. baerii*; Berg 1948b, Holčík et al. 1989, Pirogovsky et al. 1989, Shubina et al. 1989, Vlasenko et al. 1989a, b, Sokolov & Vasilev 1989a–c, Ruban 1997 this volume), as well as Amur River endemics (*A. schrenckii* and *Huso dauricus*; Artyukhin 1994, Krykhtin & Svirskii 1997 this volume), and, possibly, an Adriatic species *A. naccarii* (Tortonese 1989, Rossi et al. 1991). The origin of Ponto-Caspian species might have been associated with brackish-water derivatives of the Tethys Sea. The oldest extinct forms of the Ponto-Caspian group from the Upper Cretaceous occur in Central Asia (Nesov & Kaznyshkin 1983).

The China-western American group consists of *Acipenser sinensis*, *A. dabryanus*, *A. medirostris*, and *A. transmontanus*; the external morphology of the last two species is very similar (Findeis 1993). All of these species seem to have common Tertiary roots (Artyukhin & Andronov 1990). The two extant paddlefish species also indicate a trans-Pacific pattern that is strengthened by the inclusion of fossil taxa, such as Eocene *Crossopholis* and Paleocene *Paleopsephurus* (Grande & Bemis 1991, Jin 1995, Bemis et al. 1996, Grande & Bemis 1996).

The third group includes European and American Atlantic sturgeons (Vladykov & Greeley 1963, Kinzelbach 1987, Holčík et al. 1989). Probably, the European *A. sturio* has many primitive characters of the genus (Nesov & Kaznyshkin 1977). Once considered a subspecies of *A. sturio*, the American Atlantic sturgeons were subsequently split off as a separate species, *A. oxyrinchus* (Vladykov & Greeley 1963). Then, two subspecies, *A. o. oxyrinchus* and *A. o. desotoi*, were described within *A. oxyrinchus* (Vladykov 1955, Vladykov & Greeley 1963). The origin and spread of the Atlantic sturgeon probably reflect close Tertiary links between Europe and North America (Artyukhin & Andronov 1990). The freshwater sturgeons of eastern North America, belonging to the fourth group, the lake sturgeon, *A. fulvescens*, and shortnose sturgeon, *A. brevirostrum*, are possibly closely related (Vladykov & Greeley 1963) and may have originated on the Eastern coast of North America (Artyukhin & Andronov 1990).

Evidence for monophyly of these four groups remains uncertain, and the relationships among them are unknown. Recently, Artyukhin (1995) published a first phylogenetic tree based on general data of morphology, biogeography and karyology (but not the DNA content) of *Acipenser*. Atryukhin's scheme is the first modern attempt to reconstruct relationships within the genus *Acipenser* (see Bemis et al. 1997, for a history of the 19th century attempts to subdivide *Acipenser* into subgenera).

Artyukhin & Andropov (1990) wrote: 'It is quite evident that methods of biochemical genetics and karyology, as well as paleontological data and cur-

Table 1. Chromosome numbers and DNA content in the Acipenseriformes, Lepisosteiformes, and Amiiformes[1].

Species	Chromosome numbers	DNA content in pg	Ploidy, n (according to the DNA content)	Reference
Order Acipenseriformes				
family **Polyodontidae**				
North America				
Polyodon spathula				
Tennesse River, Alabama	120	–	–	Dingerkus & Howell (1976)
Kentucky	–	3.9[2]	4	Tiersch et al. (1989)
Missouri	–	4.89[2]	4	Blacklidge & Bidwell (1993)
Moscow Aquarium, Russia	–	3.17[2]	4	Birstein et al. (1993)
family **Acipenseridae**				
subfamily **Acipenserinae**				
Europe				
Acipenser gueldenstaedtii				
Volga River	250 ± 8	–	–	Birstein & Vasiliev (1987)
		7.87[2]	8	Birstein et al. (1993)
Caspian Sea	247 ± 8	–	–	Vasiliev (1985)
Sea of Azov	250 ± 8	–	–	Vasiliev (1985), Arefjev (1989a)
Italy, cell culture	256 ± 8	–	–	Fontana et al. (1995)
A. naccarii				
Italy	239 ± 7	–	–	Fontana & Colombo (1974)
	246 ± 8	–	–	Fontana (1994)
Italy, cell culture	246 ± 8	–	–	Fontana et al. (1995)
A. nudiventris				
Black Sea	118 ± 2	–	–	Arefjev (1983), Vasiliev (1985)
Aral Sea, Uzbekistan (Central Asia)	–	3.90[2]	4	Birstein et al. (1993)
A. persicus				
Caspian Sea	> 200	–	–	Fashkhami (pers. comm.)
A. ruthenus				
Volga River	118 ± 2	–	–	Vasiliev (1985), Birstein & Vasiliev (1987)
	–	3.74[2]	4	Birstein et al. (1993)
Don River	118 ± 3	–	–	Arefjev (1989b)
Danube, Yugoslavia	116 ± 4	–	–	Fontana et al. (1977)
Danube, Slovakia	118 ± 3	–	–	Rab (1986)
Italy (aquaculture)	118 ± 4	–	–	Fontana (1994)
Italy, cell culture	118 ± 9	–	–	Fontana et al. (1995)
A. stellatus				
Volga River	118 ± 2	–	–	Birstein & Vasiliev (1987)
	–	3.74[2]	4	Birstein et al. (1993)
A. sturio				
Italy	116 ± 4	–	–	Fontana & Colombo (1974)
	–	3.6[4]	4	Fontana (1976)
	–	3.2[6]	4	Mirsky & Ris (1951)
Huso huso				
Don River	118 ± 2	–	–	Serebryakova et al. (1983), Arefjev (1989b)
Volga River	118 ± 2	–	–	Birstein & Vasiliev (1987)
	–	3.17[2]	4	Birstein et al. (1993)
Italy	116 ± 4	–	–	Fontana & Colombo (1974)
	–	3.6[4]	4	Fontana (1976)
Asia				
Siberia				
Acipenser baerii				
Lena River	248 ± 5	–	–	Vasiliev et al. (1980)
Italy (aquaculture)	246 ± 8			Fontana (1994)

Table 1. (Continued).

Species	Chromosome numbers	DNA content in pg	Ploidy, n (according to the DNA content)	Reference
Far East and China				
A. mikadoi (*A. medirostris mikadoi*)				
Tumnin (Datta) River	[500?][3]	14.20[2]	16	Birstein et al. (1993)
A. schrenckii				
Amur River	[240?][5]	–	–	Sereberyakova (1970)
A. sinensis				
Yangtze River	264 ±	–	–	Yu et al. (1987)
Huso dauricus				
Amur River	[120?][5]	–	–	Serebryakova (1969)
	–	3.78[2]	4	Birstein et al. (1993)
North America				
A. brevirostrum				
Florida and South Carolina, USA	[360? or 500?][3]	13.08[2]	12 (?) or 16	Blacklidge & Bidwell (1993)
A. fulvescens				
Wisconsin	[250?][3]	8.90[2]	8	Blacklidge & Bidwell (1993)
A. medirostris				
Washington	[250?][3]	8.82[2]	8	Blacklidge & Bidwell (1993)
A. oxyrinchus desotoi				
Florida	[120?][3]	4.55[2]	4	Blacklidge & Bidwell (1993)
A.o. oxyrinchus				
Halifax, cell culture	(99–112)[7]	–	–	Li et al. (1985)
A. transmontanus				
San Francisco Bay, California, cell culture	(230 ±)[8]	–	–	Hedrick et al. (1991)
	–	9.55[2]	8	Blacklidge & Bidwell (1993)
Snake River, Idaho	–	9.12[2]	8	Blacklidge & Bidwell (1993)
Columbia River, Washington	–	9.59[2]	8	Blacklidge & Bidwell (1993)
Italy (aquaculture)	248 ± 8	–	–	Fontana (1994)
subfamily **Scaphirhynchinae**				
Central Asia				
Pseudoscaphirhynchus kaufmanni				
Amu Darya River, Uzbekistan	[120?][3]	3.47[2]	4	Birstein et al. (1993)
North America				
Scaphirhynchus platorynchus				
Illinois	112 ±	3.6[2]	4	Ohno et al. (1969)
Order Lepisosteiformes				
family **Lepisosteidae**				
North America				
Lepisosteus oculatus	68 ±	2.8[4]	2	Ohno et al. (1969)
L. osseus	56	–	–	Ojima & Yamano (1980)
L. platostomus	54	–	–	Ueno (1985), cit. in Suzuki & Hirata (1991)
Order Amiiformes				
family **Amiidae**				
North America				
Amia calva	46 ±	2.4[4]	2	Ohno et al. (1969)
		2.3[6]	2	Mirsky & Ris (1951)
	46	2.0[4]	2	Suzuki & Hirata (1991)

[1] All species investigated karyologically so far are listed; the DNA content values for all species studied are given.
[2] Determined by flow cytometry.
[3] Chromosome number is assumed on the basis of the DNA content.
[4] Determined by microdensitometry of Feulgen-stained nuclei.
[5] Only macrochromosomes were counted precisely.
[6] Determined by the biochemical Schmidt-Thankauser method.
[7] Chromosome number was determined in cardiac tissue cell culture.
[8] Chromosome number was determined in cell cultures: the modal 2n in a spleen cell line was 219, and in a heart cell line, 237–243 (Hedrick et al. 1991).

Table 2. Characteristics of karyotypes of several acipenseriform species[1].

Species	Chromosome number	Number of large metacentrics plus medium meta/submetacentrics, M + m/sm	Number of large telocentrics	Number of microchromosomes	Approximate arm number, NF	Reference
1. Tetraploid species						
Family **Polyodontidae**						
Polyodon spathula	120	8 + 36	4	72	164	Dingerkus & Howell (1976)
Family **Acipenseridae**						
Acipenser nudiventris						
Black Sea, Russia	118 ± 3	8 + 46	4	60 ± 3	172	Arefjev (1983)
A. ruthenus						
Sea of Azov, Russia	118 ± 3	8 + 50	4	56 ± 3	176	Arefjev (1989a)
Volga River, Russia	118 ± 2	8 + 50	4	56 ±23	176	Birstein & Vasiliev (1987)
Danube, Slovakia	118 ± 4	8 + 50	4	56 ± 4	176	Rab (1986)
Danube, Yugoslavia	116 ± 2	8 + 48	4	56 + 4	170	Fontana et al. (1977)
A. stellatus						
Volga River, Russia	118 ± 2	8 + 48	4	58 ± 2	174	Birstein & Vasiliev (1987)
A. sturio						
Italy	116 ± 4	8 + 48	4	56 ± 4	172	Fontana & Colombo (1974)
Huso huso						
Volga River, Russia	118 ± 2	8 + 54	4	52 ± 2	180	Birstein & Vasiliev (1987), Arefiev & Nikolaev (1991)
Sea of Azov, Russia	118 ± 3	8 + 54	4	52 ± 3	180	Serebryakova et al. (1983), Arefiev (1989b)
Italy	116 ± 4	8 + 52	4	52 ± 4	176	Fontana & Colombo (1974)
Scaphirhynchus platorynchus						
USA	112 ± 2	8 + 52	4	48 ±	172	Ohno et al. (1969)
2. Octoploid species						
Family **Acipenseridae**						
Acipenser baerii						
Lena River, Siberia, Russia	248 ± 5	16 + 42		190	308	Vasiliev et al. (1980)
A. gueldenstaedtii						
Volga River, Russia	250 ± 8	16 + 76		155	339	Birstein & Vasiliev (1987)
Sea of Arzov, Russia	250 ± 8	16 + 82		152	348	Arefjev (1989a)
A. naccarii						
Italy (wild)	239 ± 7	16 + 76		147	331	Fontana & Colombo (1974)
Italy, aquaculture	241 ± 3	16 + 74		151	331	Arlati et al. (1995)
A. sinensis						
China	264 ± 3	16 (?) + 82		166	362	Yu et al. (1987)

[1] A revision of data from papers mentioned as References. The numbers of m/sm, microchromosomes, and NF are given approximately, since it is impossible to discriminate the form and exact number of small chromosomes and microchromosomes. For octoploid species, the number of telocentrics and microchromosomes is given together because there is no clear size difference between these two kinds of chromosomes. Usually there are 2–5 middle and/or small-sized telocentric pairs in these karyotypes.

rent views on paleogeography will provide useful tools for resolving complex relationships and phylogeny of sturgeons'. In the first part of this paper we review all cytogenetic data available on Acipenseriformes and make some new conclusions relevant to the four groups within *Acipenser* mentioned above. In the second part we describe experimental data on the molecular phylogeny of Acipenseriformes. Because multiple gene regions have been useful in other groups of fishes (reviews in Normark et al. 1991, Stock et al. 1991a, Meyer 1993, Patterson et al. 1993), we believed that they might also provide reasonable character state information for acipenseriforms. Consequently we amplified and sequenced partial fragments of 18S rRNA, 12S rRNA, 16S rRNA, and cytochrome *b* genes of a few representatives of all lineages of this order. We included the phylogenetic analysis of the combined molecular and morphological data for all the species we studied. Also, we examined relationships among representatives of four species groups of the genus *Acipenser* recognized by Artyukhin (1995), using data for a partial sequence of the cytochrome *b* gene.

Acipenseriform cytogenetics: an overview

Main karyotypic characteristics, DNA content, and polyploidy

Karyotypes of about half of all sturgeon species have been described and the DNA content in most sturgeon species and American paddlefish has been measured (Table 1). Acipenseriform karyotypes investigated so far have two particular characteristics: (1) they are large; and (2) they consist of macro- and microchromosomes. According to the number of chromosomes (2n), the species can be divided into two groups: those with about 120 chromosomes (e.g., *Huso huso*, *H. dauricus*, *Acipenser ruthenus*, *A. stellatus*, *A. nudiventris*, *A. sturio*, and *Polyodon spathula*), and those with 240 chromosomes (e.g., *A. gueldenstaedtii*, *A. naccarii*, *A. baerii*, *A. schrenckii*, and *A. transmontanus*). By comparison to the 120-chromosome species, the 240-chromosome species are tetraploids.

The size of macrochromosomes in both groups is between 2–5 μm, and the majority of macrohromosomes are the meta- and submetacentrics (Table 2). One third to one half of the chromosome number in both groups is comprised of microchromosomes of a very small size (about 1 μm). Karyotypes of the 120-chromosome species typically consist of 4 pairs of large metacentrics (no. 1–4), 5 pairs of large but somewhat smaller metacentrics (no. 5–9), about 20 pairs of medium-sized metacentrics and/or submetacentrics of gradually decreasing size (no. 10–30), one pair of comparatively large telocentrics (no. 30), one pair of small telocentrics, and approximately 56 ± 4 microchromosomes of different form (Table 2). The difference between karyotypes of representatives of two lineages of the extant acipenseriforms, the Polyodontidae (*Polyodon spathula*) and Acipenseridae (all other 120-chromosome species in Table 1), as well as of the lineages within the Acipenseridae (*Huso huso*, the 120-chromosome species of the genus *Acipenser*, and *Scaphirhynchus platorynchus*), seems to be small. Evidently, the ancestral acipenseriform karyotype was preserved in these fishes without dramatic changes during diverisification of the group.

In general, few karyotypic changes are noticeable among the species of *Acipenser* with 120-chromosomes (Table 2). The karyotype of *Huso huso* is more symmetric than those of the 120-chromosome species of *Acipenser*, i.e., it contains more biarmed chromosomes and fewer microchromosomes (see Morescalchi 1973). Also, there is a small difference among the 120-chromosome species in the size of a pair of large telocentrics (no. 30): it is small in *A. sturio* (Fontana & Colombo 1974) and *A. nudiventris* (Arefjev 1983) and it is as large as pair no. 8 or 9 in *Huso huso* (Fontana & Colombo 1974, Birstein & Vasiliev 1987, Arefjev 1989b) and *A. ruthenus* (Rab 1986, Birstein & Vasiliev 1987).

The similarity of karyotypes of the 120-chromosome acipenseriforms points to a generally slow rate of karyological evolution. This correlates with a slow rate of nuclear DNA evolution: practically all genome fractions (both the repeated and unique sequences) are homologous in *Acipenser ruthenus*, *A. stellatus*, *A. gueldenstaedtii*, and *H. huso*, and the number of nucleotide substitutions in the first frac-

tion is 0–2.65, and 1.5–2.7% in the second (Kedrova et al. 1980). These data were supported by our results from sequencing the 18S genes, which are almost invariable among acipenseriforms (see below).

A low degree of protein evolution is usually characteristic of the acipenseriforms, especially the 120-chromosome species. A mean heterozygosity for three freshwater species, *Polyodon spathula*, *Scaphirhynchus platorynchus* and *S. albus*, is between 0.010 and 0.017 (Carlson et al. 1982, Phelps & Allendorf 1983) and is the highest (of all species investigated) in the anadromous *Acipenser stellatus*, 0.093 (Ryabova & Kutergina 1990). All of these species are 120-chromosome forms. The mean heterozygosity for other osteichthyans is 0.051 (Ward et al. 1992). The complete lack of genetic divergence at the protein level between the two species of *Scaphirhynchus* is very unusual for fishes, especially because freshwater fishes typically exhibit subpopulational differentiation significantly higher than that of anadromous and especially marine species (Ward et al. 1994).

The DNA content (2C) of all of the 120-chromosome species is about 3.7–3.9 pg (in *Polyodon spathula* it is a little lower, 3.2 pg), whereas in the 240-chromosome species it is twice as high, 7.9–8.3 pg (Birstein et al. 1993, Table 1). Moreover, the same tendency in DNA content is present in American sturgeons whose karyotypes have not been investigated: *A. oxyrinchus desotoi* (2C = 4.6 pg) is evidently a 120-chromosome subspecies, whereas *A. fulvescens* and *A. medirostris* (American form) are 240-chromosome species, 2C = 8.8–8.9 pg (Blacklidge & Bidwell 1993; the slightly higher DNA content for all American species as compared with the Eurasian species in Table 1 is due to the different methods used by Birstein et al. 1993, and Blacklidge & Bidwell 1993). DNA content in *Pseudoscaphirhynchus kaufmanni* (3.2 pg) is slightly lower than in the sterlet, *A. ruthenus* (3.7 pg), which was used by Birstein et al. (1993) as a standard species for comparative measurments due to its invariable DNA content. However, this difference is so small that we predict *P. kaufmanni* is a 120-chromosome species.

The sterlet, *Acipenser ruthenus*, has other properties which attest to its genetic stability. The basic chromosome number in three generations of the 'bester', the fertile hybrid between beluga, *Huso huso*, and sterlet, *A. ruthenus*, does not differ from that of parental species, and a gradual displacement of karyotypic parameters (numbers of bi- and uniarmed chromosomes) towards those of the sterlet occurs (Arefjev 1989b). The sterlet has evidently not only invariable DNA content, but also a dominant karyotype. The situation is very unusual, because, as a rule, the karyotypes of fish interspecies hybrids are more variable than karyotypes of the parental species (Arefjev 1991, Arefjev & Filippova 1993).

The Asian green (Sakhalin) sturgeon, *A. mikadoi* (or *A. medirostris mikadoi* as explained below), and the American shortnose sturgeon, *A. brevirostrum*, have even higher DNA contents than the 240-chromosome forms. The DNA content of *A. mikadoi* is 14.2 pg per nucleus, four times higher than in the 120-chromosome species or roughly twice that of the 240-chromosome forms (Birstein et al. 1993). In American green sturgeon, *A. medirostris*, it is about half of this value (8.8 pg, Blacklidge & Bidwell 1993). Therefore, the American green sturgeon seems to be a typical 240-chromosome form, while the Sakhalin sturgeon might be predicted to have twice the number of chromosomes, or around 500. If so, the Sakhalin sturgeon would have the highest diploid number reported in vertebrates. But polyploidization can occur without an increase in chromosomes number, as in several species of sharks (see below), and only direct karyotypic study can address the chromosome number and morphology of the Sakhalin sturgeon. DNA content data support the old point of view that the Asian form of *A. medirostris* is a separate species, *A. mikadoi* (Hilgendorf, 1892), or a subspecies, *A. medirostris mikadoi* (Shmidt 1950, Lindberg & Legeza 1965; in Table 1 it is mentioned as a species, see Birstein 1993b). Although Blacklige & Bidwell (1993) consider *A. brevirostrum* to be an allopolyploid (12n = 360), a descendant of ancestral spontaneous triploids, allopolyploidy is unknown in other acipenserids, and it is more logical to propose that this is a 16n-ploid.

An investigation of active nucleoli gave additional information about ploidy in the acipenseriforms. There are different modal numbers of nucleoli per

nucleus in species investigated (Table 3): 2–4 (maximum 6) in the 120-chromosome species, and 6–8 (maximum 11–12) in the 240-chromosome species of *Acipenser* (Birstein & Vasiliev 1987, Fontana 1994). In *A. ruthenus*, 4n, the NORs are located in two pairs of small chromosomes, a pair of metacentrics and a pair of telocentrics (possibly no. 30; Rab 1986, Birstein & Vasiliev 1987, Fontana 1994). According to Arefjev (1993), in octoploid *A. baerii* NORs are located also on two pairs of chromosomes, while Fontana (1994) found two quadruplets bearing NORs in *A. baerii*, *A. naccarii*, and *A. transmontanus*. The average number of nucleoli in *Polyodon spathula* is 4 (Dingerkus & Howell 1976), as many as in the 120-chromosome species of *Acipenser* (Table 3). Usually the number of nucleoli in diploid teleosts is half that of the 120-chromosome acipenseriforms and equals 1–2 nucleoli per nucleus (review in Birstein 1987).

The tendency seen in 120-chromosome sturgeons to have more nucleoli than in diploid teleosts is apparently caused by their high ploidy. Ohno et al. (1969) arranged the first 64 chromosomes of *Scaphirhynchus platorynchus*, 4n = 116 ± into 16 groups of four homologs, and Dingerkus & Howell (1976) divided the karyotype of 120-chromosome *Polyodon spathula* into 30 groups consisting of four chromosomes of similar morphology. If it is taken into consideration that this species has 4 active nucleoli per nucleus (see above), it is clear that *P. spathula* is a tetraploid. The same procedure of chromosome grouping (but with greater uncertainity) can be done for *A. ruthenus* and *H. huso* (Birstein & Vasiliev 1987). From this comparison, one can conclude that the 120-chromosome species are in reality tetraploids, while the 240-chromosome species are really octoploids, and the ploidy of *A. mikadoi* and *A. brevirostrum* possibly is 16n.

Further evidence that the 120-chromosome species are tetraploids comes from the existence of duplicated loci, a common characteristic of polyploids. A high level of duplicated loci (31%) was found in *A. stellatus* (Nikanorov et al. 1985, Ryabova & Kutergina 1990); duplicated loci were also found in *Huso huso* (Slynko 1976). In *A. stellatus*, duplicated loci Ldh3 and Ldh4 are located at two different chromosomes (Kutergina & Ryabova 1990). In *Polyodon spathula* the expression of dupli-

Table 3. Number and location of nucleolar organizer regions (NORs) in Acipenseriformes (data on Ag-staining).

Species	Chromosome number	Number of NORs per nuclei[1]	Number of NORs-bearing chromosomes and NORs' location[2]	Reference
Family **Polyodontidae**				
Polyodon spathula	120	4		Dingerkus & Howell (1976)
Family **Acipenseridae**				
1. Tetraploid species				
Acipenser ruthenus	118	2–3 (1–6)	Two pairs (T & m), telomeric	Birstein & Vasiliev (1987)
	118	–	Two pairs, telomeric	Fontana (1994)
A. stellatus	118	2–3 (1–6)	Two pairs (both (?) m), telomeric	Birstein & Vasiliev (1987)
Huso huso	118	2–3 (1–6)	Two pairs (both (?) m), telomeric	Birstein & Vasiliev (1987)
2. Octoploid species				
Acipenser baerii	250	4 (2–6)	Two pairs (T & m)	Arefjev (1993)
	250	–	Two quadruplets	Fontana (1994)
A. gueldenstaedtii	250	6–8 (2–12)	–	Birstein & Vasiliev (1987)
A. naccarii	246	–	Two quadruplets	Fontana (1994)
A. transmontanus	248	–	Two quadruplets	Fontana (1994)

[1] The modal number; a variation in the number is given in the parenthesis.
[2] T means medium-sized telocentric, and m, microchromosome; telomeric means telomeric location of NORs.

Table 4. Natural hybridization of sturgeon species and their ploidy.

Interspecies hybrids	Intergenera hybrids
1. Caspian Sea basin[1]	
(a) Volga River	
A. ruthenus (4n) × *A. stellatus* (4n)	*H. huso* (4n) × *A. gueldenstaedtii* (8n)
A. stellatus (4n) × *A. ruthenus* (4n)	*H. huso* (4n) × *A. ruthenus* (4n)
A. nudiventris (4n) × *A. gueldenstaedtii* (8n)	
A. gueldenstaedtii (8n) × *A. ruthenus* (4n)[2]	
A. gueldenstaedtii (8n) × *A. stellatus* (4n)	
A. gueldenstaedtii (8n) × *A. persicus* (8n)[3]	
(b) Kama River	*Huso huso* (4n) × *A. nudiventris* (4n)
	H. huso (4n) × *A. gueldenstaedtii* (8n)
	H. huso (4n) × *A. stellatus* (4n)
	A. ruthenus (4n) × *H. huso* (4n)
(c) Ural River	
A. nudiventris (4n) × *A. stellatus* (4n)	*H. huso* (4n) × *A. stellatus* (4n)
A. stellatus (4n) × *A. nudiventris* (4n)	
(d) Kura River	
A. nudiventris (4n) × *A. stellatus* (4n)	*H. huso* (4n) × *A. nudiventris* (4n)
A. stellatus (4n) × *A. nudiventris* (4n)	
A. nudiventris (4n) × *A. gueldenstaedtii* (8n)	
(e) Sefir-Rud River	
A. nudiventris (4n) × *A. stellatus* (4n)	
A. nudiventris (4n) × *A. gueldenstaedtii* (8n)	
(f) Caspian Sea	*H. huso* (4n) × *A. persicus* (8n)
2. Sea of Azov basin[4]	
Don River	
A. ruthenus (4n) × *A. stellatus* (4n)	
3. Black Sea basin[5]	
(a) Danube	
A. ruthenus (4n) × *A. stellatus* (4n)	*A. gueldenstaedtii* (8n) *H. huso* (4n)
A. ruthenus (4n) × *A. nudiventris* (4n)	*A. stellatus* (4n) × *H. huso* (4n)
A. stellatus (4n) × *A. ruthenus* (4n)	*A. nudiventris* (4n) × *H. huso* (4n)
A. ruthenus (4n) × *A. gueldenstaedtii* (8n)	*H. huso* (4n) × *A. stellatus* (4n)
A. stellatus (4n) × *A. gueldenstaedtii* (8n)	
A. nudiventris (4n) × *A. gueldenstaedtii* (8n)	
A. sturio (4n) × *A. gueldenstaedtii* (8n)	
(b) Black Sea	
A. gueldenstaedtii (8n) × *A. sturio* (4n)	
A. gueldenstaedtii (8n) × *A. nudiventris* (4n)	
4. Siberian rivers	
Main rivers (Yenisey, Lena, Ob, Kolyma)[6]	Amur River[7]
A. baerii (8n) × *A. ruthenus* (4n)	*Huso dauricus* (4n) × *A. schrenckii* (8n?)
5. Central Asia[8]	
Amu-Darya River	
Pseudoscaphirhyncus kaufmanni (4n) × *P. hermanni*	
6. North America[9]	
Missouri and Mississippi Rivers	
Scaphirhynchus albus (?) × *S. platorynchus* (4n)	

[1] Data from Berg (1911, 1948b), Kozhin (1964), Kozlov (1970), Legeza (1971), and Keyvanfar (1988).

[2] In the early 1950s, this hybrid was the most numerous (46% of all hybrids caught; Konstantinov et al. 1952).

[3] Data from Vlasenko et al. (1989b).

[4] Data from Berg (1948b) and Kozhin (1964).

[5] Data from Antipa (1909), Antoni-Murgoci (1946), Banarescu (1964), and Berg (1948b).

[6] Data from Berg (1948b).

[7] Data from Berg (1948b), Wei et al. (1996), and Krykhtin & Svirskii (1996).

[8] Data from Nikolskii (1938) and Berg (1948b).

[9] Data from Carlson et al. (1985).

cated loci is much lower, only 6% (Carlson et al. 1982). Why this is so is unknown. In this species duplicated loci for insulin, glucagon and glucagon-like peptide were found (Nguyen et al. 1994).

As for the 240-chromosome octoploid species, it is evident that two forms of vitellogenin monomers in American *A. transmontanus* (Bidwell et al. 1992) and two forms of growth hormones in Russian *A. gueldenstaedtii* (Yasuda et al. 1992) are a result of polyploidization. A higher ploidy level seems to be a reason for a higher heterozygosity in *A. gueldenstaedtii* compared to the 120-chromosome species of *Acipenser* from the same geographic area (Slynko 1976, Keyvanfar 1988, Kuzmin 1991). Possibly, a high level of ploidy causes a high variation in the mean heterozygosity of American octoploid *A. transmontanus* (0.014–0.069, Bartley et al. 1985). Hemoglobin, the only protein examined in the 16n-ploid *A. mikadoi*, is more heterogeneous (11 electrophoretic fractions) than in tetra- (8–9 fractions) or octoploid (7–8 fractions) species of *Acipenser* (Lukyanenko & Lukyanenko 1994).

Polyploidization is a relatively uncommon genetic mechanism in vertebrates, occurring only in lampreys, elasmobranchs, acipenseriforms, some groups of teleosts (salmonids, cyprinids, and catostomids), amphibians (anurans), and lizards (review in Birstein 1987). To date, polyploidization is unknown in birds or mammals. It seems that the polyploid state, and karyotypic and genomic similarity of different acipenseriforms contribute to easy interspecific and even intergeneric hybridization within the Acipenseridae.

The Acipenseridae is the only group among vertebrates all members of which can hybridize with each other in the wild if their spawning grounds overlap (Table 4). The unique easiness of hybridization of acipenserids was described by Russian ichthyologists more than 100 years ago (Ovsyannikov 1870, Zograf 1887). Some hybrids (such as the artificially obtained 'bester', *H. huso* × *A. ruthenus*, and its reciprocal hybrid, Nikolyukin 1970), have the desirable characteristics of fast growth and high viability, are fertile (which is also unusual for vertebrate hybrids) and are widely used in aquaculture (Williot et al. 1993). Besters inherit a phenotype intermediate between the parental species. Of 29 characters studied, 9 deviated toward the maternal species (beluga), and 18 deviated toward the paternal species (sterlet) (Krylova 1981). The meristic characters (the number of dorsal, lateral, and ventral scutes) deviated toward the maternal species (beluga). The ease of hybridization of sturgeon species allowed to show the maternal inheritance of some behavior characters of sturgeons (Marshin et al. 1969).

Problem of the ancestral karyotype

Data presented above support the hypothesis of the tetraploid origin of 120-chromosome acipenseriforms from a 60-chromosome ancestor before the radiation of this order (Dingerkus & Howell 1976, Carlson et al. 1982). The karyotypes of Lepisosteidae (gars) and Amiidae (bowfins) are relevant to understanding the proposed acipenseriform ancestral karyotype. Gars have approximately 60 chromosomes (Table 1), but it seems that many karyological changes have occurred during their evolution. The karyotype of *Lepisosteus oculatus*, 2n = 68, consists of many meta- and acrocentric macrochromosomes, as well as many microchromosomes (Ohno et al. 1969), while the karyotype of *L. osseus*, 2n = 56, lacks microchromosomes (Ojima & Yamano 1980). The karyotype of *Amia calva* is even more reduced, 2n = 46, but it still includes microchromosomes (Ohno et al. 1969, Suzuki & Hirata 1991). The cellular DNA content of gars and *Amia* ranges from 2.0 to 2.8 pg per nucleus, which is approximately half that of the 120-chromosome acipenseriforms. Therefore, it is quite possible that the common ancestor of acipenseriforms and neopterygians had a karyotype of about 60 chromosomes consisting of micro- and macrochromosomes, with a DNA content about 2.0 pg per nucleus.

Extant polypterids, which are the living members of the basal actinopterygian group Cladistia, have 36 bi-armed chromosomes (except *Polypterus weekesii*, 2n = 38; reviews in Vervoort 1980, Suzuki et al. 1988, 1989). The DNA content in polypterids is considerably higher than in the acipenseriforms, 2C = 12–13 pg per nucleus (Vervoort 1980). It is evident that the polypterids are a cytogenetically advanced

group. Molecular data also support this conclusion: the 18S rRNA sequences in the species of the genera *Polypterus* and *Erpetoichthys* are very similar to each other, but both are highly divergent from those of other gnathostomes (Stock et al. 1991a).

The karyotypes of chondrichthyans are more informative. Chondrichthyans are mostly tetraploids, 4n = 90–104 (reviews in Schwartz & Maddock 1986, Asahida et al. 1988, 1993, Asahida & Ida 1989, 1990, Stingo et al. 1989, Stingo & Rocco 1991), and karyotypes contain macro- and microchromosomes. According to cellular DNA content and DNA re-association kinetics data (Olmo et al. 1982, Ida et al. 1985), the ploidy level of a few species of sharks is higher, and in these cases polyploidization occurred without a change in the chromosome number (phenomenon known as cryptopolyploidy; Wagner et al. 1993). The only extant chondrichthyan which possibly retains a diploid karyotype is the spotted ratfish, *Hydrolagus colliei*: it has the lowest chromosome number among elasmobranchs, 2n = 58, and the lowest DNA content, 2C = 3.0 pg (Ohno et al. 1969). But this could be a derived condition, for insufficient taxa have been studied to draw any conclusion. Moreover, changes in DNA content occur even during ontogenesis of this species: about 10% of the genome is eliminated in somatic tissues as compared with the germ cells (Stanely et al. 1984).

In contrast to Acipenseriformes, the reduction of chromosome number through fusion of micro- and small chromosomes into macrochromosomes was the main evolutionary karyotypic trend in chondrichthyans (Schwartz & Maddock 1986, Stingo et al. 1989). As a result, the karyotypes of advanced chondrichthyans consist of a smaller number of mainly bi-armed chromosomes, 2n = 50–70. Another considerable difference is that acipenseriforms have numerous bi-armed macrochromosomes, whereas the most generalized, plesiomorphic elasmobranchs have only 2–3 pairs of bi-armed macrochromosomes, and up to 50 pairs represented by small telocentrics or by microchromosomes (Schwartz & Maddock 1986, Stingo & Rocco 1991). Moreover, the average DNA content in elasmobranchs is much higher than in acipenseriforms (reviews in Schwartz & Maddock 1986, Birstein 1987, Asahida et al. 1988, 1993). Based on general characteristics of the karyotypes of spotted ratfish, gars, and sturgeons, Ohno (1970) and later Dingerkus (1979) proposed that the ancestral karyotype of gnathostomes consisted of approximately 50–60 macro- and microchromosomes.

Recently a karyotype consisting of macro- and microchromosomes was described in another living fossil fish, the coelacanth *Latimeria chalumnae* (Bogart et al. 1994). It appears to include 16 pairs of macro- and eight pairs of microchromosomes. This karyotype resembles to a high extent that of one of the most primitive living frogs, *Ascaphus truei*, but this resemblance could be coincidental.

Karyotypes of acipenseriforms generally resemble karyotypes of primitive amphibians, not anurans as in *L. chalumnae*, but, instead, urodeles belonging to the family Hynobiidae. With several exclusions, the karyotypes of hynobiids, 2n = 56–62, consist of a few large and middle-sized pairs of bi-armed macrochromosomes, a few pairs of telocentric macrochromosomes, and 15–20 pairs of microchromosomes (Morescalchi et al. 1979, King 1990, Kohno et al. 1991). It seems that the ancestral karyotype of these amphibians consisted of 60 macro- and microchromosomes. Because the karyotypes of hynobiids, as well as those of acipenseriforms, include a large number of bi-armed macrochromosomes, they should be considered derived (as compared, for instance, with those of the most ancient forms of chondrichthyans).

Cytogenetic data and phylogeny of the Acipenseriformes

It is impossible to infer generic interrelationships within the Acipenseridae from cytogenetic data. Divergence of the three lines within the family occurred without polyploidization, and the ancestors of all three lineages within the acipenserids seem to have been tetraploids, 4n = 120. If the genus *Huso* originated as the first outshoot within the Acipenseridae (as proposed by Findeis 1993, 1997), then this event was not accompanied by a substantial karyotypic change. The ancestral karyotype seems to have been retained without significant modification, since the karyotype of *Huso huso* is only slight-

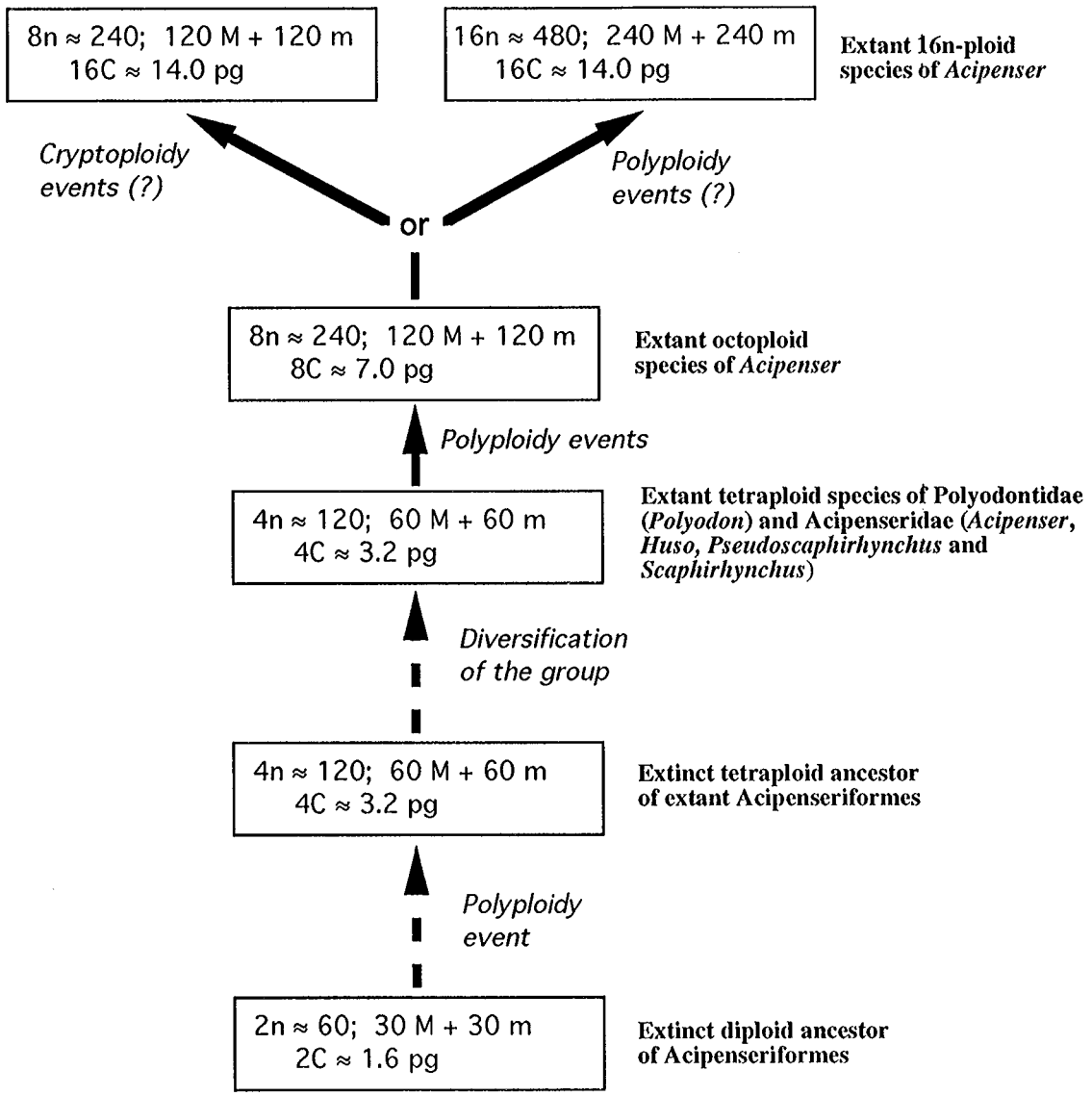

Figure 1. A schematic representation of changes in ploidy in Acipenseriformes. M = macro-, m = microchromosomes.

ly more symmetric than karyotypes of other acipenserids. A schematic course of possible cytogenetic evolution within the Acipenseriformes is presented in Figure 1.

Cytogenetic data are helpful for understanding some relationships within the genus *Acipenser*, where polyploidization was one of the main genetic mechanisms of speciation. The diversification of this genus was accompanied by an appearance of octoploid (according to the karyotypic and DNA content data) and 16n-ploid (according to DNA content data) species. The octoploid 240-chromosome sturgeon species seem to have originated independently in different regions. The closely related *A. gueldenstaedtii* and *A. persicus* may have a common origin and a common octoploid ancestor. Molecular data point to the close relatedness of *A. gueldenstaedtii* to *A. baerii* (see below).

Acipenser medirostris and *A. transmontanus* have many similar characteristics in morphology,

biology, and ecology, as well as overlapping ranges in western North America (Hart 1973, Scott & Grossman 1973). Moreover, according to Artyukhin & Andronov (1990), some ecological and biological characteristics are common to these two species and the Chinese sturgeon, *A. sinensis*. All three species are octoploid (Table 1). Possibly, tetraploidization occurred in the ancestor of all three species. If the difference between the karyotype of *A. sinensis* and *A. transmontanus* (8n = 264 ± 3 and 230 ± or 248 ± 8, respectively is real, then it means that at least two polyploidization events took place in the ancestral 120-chromosome form. The next step of polyploidization which occurred in the 240-chromosome ancestor of *A. medirostris* and *A. mikadoi*, resulted in the formation of the genome of *A. mikadoi* (which is, therefore, the youngest in this group of species). The Amur River sturgeon, *A. schrenckii*, also seems to be an octoploid (but these data are preliminary, see Table 1), as the species of the trans-Pacific *A. sinensis-A. medirostris-A. transmontanus* group, and lives in a close geographic area. According to Artyukhin (1994, 1995), *A. schrenckii* is closely related to the Ponto-Caspian species *A. nudiventris* and *A. ruthenus*.

It is difficult to draw any conclusions concerning the ancestor of *A. brevirostrum*, the species with the second highest level of DNA content. The other species of the Eastern Coast of North America, *A. oxyrinchus*, is closely related to the European 120-chromosome Atlantic sturgeon, *A. sturio*, and, according to the DNA content, has the same ploidy (Table 1). Possibly, *A. brevirostrum* is also related to some European sturgeons (*A. nudiventris*; see below), and originated from these European-related ancestors through polyploidization. Because of its high ploidy level, *A. brevirostrum* might be a young species among East American representatives of the genus *Acipenser*. As for another American species, the freshwater lake sturgeon, *A. fulvescens*, which is also an octoploid like *A. oxyrinchus* (according to its DNA content), its origin and relationships with the other species are still unclear, although it is morhologically similar to *A. brevirostrum* (Vladykov & Greeley 1963, Findeis 1993). Therefore, the cytogenetic data provide additional information (ploidy level can be considered as a character in cladistic terms) to the hypothetical grouping of species within the genus *Acipenser* based on the species morphology, ecology, biogeography and possible area of origin (Artyukhin & Andronov 1990, Artyukhin 1994, 1995).

Among fishes, an analogous mode of speciation through multiple independent tetraploidization events is characteristic of only one group of teleosts, the family Cyprinidae (review in Buth et al. 1991). A tetraploid ancestor of two species, *Cyprinus carpio* and *Carassius auratus*, appeared through tetraploidization about 16–20 million years ago (Risinger & Larhammar 1993, Larhammar & Risinger 1994). Hexaploids in Eurasia and Africa (reviews in Vasiliev 1985, Buth et al. 1991, Golubtsov & Krysanov 1993), as well as a 16/20n-ploid Asian species *Diptychus dipogon* (Yu & Yu 1990) also were formed within Cyprinidae. Moreover, as a result of a tetraploidization event which occurred approximately 50 million years ago an ancestor of another family, Catostomidae, appeared within this group (Uyeno & Smith 1972). Catostomids are considered to have been tetraploid since then (Ferris & Whitt 1979, Ueno et al. 1988, Tsoi et al. 1989). Tetraploids were also formed in another family closely related to Cyprinidae, the Cobitidae (review in Vasiliev 1985). Only one other group of teleosts, the Salmonidae (which includes three subfamilies, Coregoninae, Thymallinae, and Salmoninae, sensu Nelson 1994) also originated from a tetraploid ancestor (Gold 1979, Allendorf & Thorgaard 1984). By contrast with the acipenseriforms, cyprinids, and catostomids, a decrease in chromosome number through centromeric fusion was characteristic for different lineages of salmonids (Vasiliev 1985, Buth et al. 1991). Therefore, a gradual increase in chromosome number through polyploidization has occurred a few times during the history of actinopterygians.

Molecular phylogeny of the Acipenseriformes

Our first objective was to search for molecular synapomorphies of Acipenseriformes and its major included clades using representatives of all extant genera (except the Chinese paddlefish, *Psephurus gladius*, due to our inability to obtain suitable tis-

sue). Our data were polarized using other Actinopterygii, *Polypterus* and *Amia*. We also attempted a synthesis of morphological, karyological and molecular characters as related to relationships among Acipenseriformes. Finally, we examined interrelationships among representatives of Artyukhins species groups proposed for *Acipenser* (see above).

Whereas our examination of acipenseriform taxa concentrated on comparisons of species belonging to different genera or species within the genus *Acipenser*, most previous workers have concentrated on intraspecies structure using the control region (D-loop) of the mtDNA. Buroker et al. (1990) showed that in the American white sturgeon, *Acipenser transmontanus*, mtDNA size varies between 16.1 and 16.7 kb depending on the number of tandemly repeated 82 nucleotide sequences in the control region of the mtDNA. Nearly 50% of the individuals studied by Brown et al. (1992a) were heteroplasmic (i.e., had multiple copies of different mtDNA types within an individual) for length variation, with six different mtDNA length variants found (Brown et al. 1992b, 1993). Fifty percent of *A. medirostris* studied were also heteroplasmic; D-loops of these individuals included from one to four repeats (Brown et al. 1996). The average size of mtDNA of the lake stugeon, *A. fulvescens*, is approximately the same as that of white sturgeon, 16.6–16.9 kb (Guénette et al. 1993, Ferguson et al. 1993) or 16.1–16.5 kb (Brown et al. 1996). No heteroplasmy was detected in *A. fulvescens* and *A. oxyrinchus* (Brown et al. 1996). All individuals of *A. fulvescens* studied had one of five possible mtDNA size variants which closely corresponded to *A. transmontanus* with one to five repeat units. In *A. oxyrinchus*, nearly every individual was fixed for mtDNA roughly equivalent in size to the smallest repeat found in the other species. Restriction analysis of mtDNA (Bowen & Avise 1990, Avise 1992) and partial sequencing of the control region (Miracle & Campton 1995, Ong et al. 1996, Wirgin et al. 1997 this volume) were used for inferring relationships between subspecies and populations of *A. oxy-*

Table 5. List of sturgeon species and blood samples studied.

Species number	Species	Geographical region	Number of blood (or tissue) samples	Collector
1.	*Acipenser baerii*[1]	Lena River, Siberia, Russia (Moscow Aquarium)	2	V. Birstein
2.	*A. brevirostrum*	Connecticut River, MA, USA	(eggs)	B. Kynard
3.	*A. gueldenstaedtii*[1]	Volga River, Russia (Moscow Aquarium)	2	V. Birstein
4.	*A. medirostris*	Columbia River, OR, USA	1	J. North
5.	*A. mikadoi*	Tumnin River, Russia	2 (fragments of muscles)	E. Artuykhin
6.	*A. naccarii*	Ferrara, Italy (Aquarium)	2	F. Fontana
7.	*A. nudiventris*[1]	Aral Sea, Uzbekistan (Moscow Aquarium)	2	V. Birstein
8.	*A. oxyrinchus oxyrinchus*	Hudson River	2 (fragments of muscles)	J. Waldman
9.	*A. ruthenus*[1]	Volga River, Russia (Moscow Aquarium)	2	V. Birstein
10.	*A. stellatus*[1]	Volga River, Russia (Moscow Aquarium)	2	V. Birstein
11.	*A. transmontanus*	Columbia River, OR, USA	2	J. North
12.	*Huso dauricus*[1]	Amur River, Siberia, Russia (Moscow Aquarium)	2	V. Birstein
13.	*Pseudoscaphirhynchus kaufmanni*[1]	Amu-Darya River, Uzbekistan (Moscow Aquarium)	2	V. Birstein
14.	*Scaphirhynchus albus*	Yellowstone River, MT, USA	2	H. Bollig
15.	*Polyodon spathula*[1]	Moscow Aquarium	1	V. Birstein

[1] These samples were used for the DNA content measurements in Birstein et al. (1993); see Table 1 above.

rinchus. Both subspecies, *A. o. oxyrinchus* and *A. oxyrinchus desotoi*, exhibited low mtDNA diversity.

The order and transcriptional polarity of three mitochondrial genes in *A. transmontanus* (genes for cytochrome *b*, threonine and proline tRNAs) are identical to those of other vertebrates (Gilbert et al. 1988, Brown et al. 1989, Buroker et al. 1990). The whole sequence of the cytochrome *b* gene for *A. transmontanus*, as well as partial sequences of the same gene for *Scaphirhynchus platorynchus* and *Polyodon spathula* were published recentely (Brown et al. 1989, Normark et al. 1991). Partial sequences of the same gene for *A. brevirostrum*, *A. oxyrinchus*, *Scaphirhynchus albus*, and *S. suttkusi* were submitted by W. Schill into GenBank under numbers Z22822, L35111, L35110, and L35112, respectively. Although acipenseriforms were included in a higher level phylogenetic analysis based on cytochrome *b* sequence (Normark et al. 1991), no direct evidence on the utility of other gene regions for phylogenetic analysis is available.

Because phylogenetic divergence within the Acipenseriformes is potentially broad, based on the fossil record (e.g., Grande & Bemis 1991, Bemis et al. 1997), no single gene region can be assumed to be adequately broadly informative on all phylogenetic levels as a source of characters. Consequently we chose to examine several gene regions as potential sources of characters, including well characterized gene regions from mitochondrial DNA (16S rDNA, 12S rDNA, and cytochrome *b*) and one nuclear gene region (18S rDNA). Below we discuss each of these four gene regions.

Materials and methods

Specimens

Species used in this study and location of the fishes are listed in Table 5. With three exceptions (*Acipenser brevirostrum*, *A. mikadoi*, and *A. oxyrinchus*), blood samples were taken, mixed with buffer (100 mM Tris, 100 mM EDTA, and 2% SDS; 0.5 ml of blood and 5 ml of buffer), and the blood cells lysed in this solution were kept in a freezer at −70° C. Most Russian specimens examined were the same individuals used for DNA content measurements by Birstein et al. (1993). Also, we isolated DNA from alcohol-fixed samples of muscles of *Amia calva* and *Polypterus senegalus* provided by Paul Vrana (American Museum of Natural History, New York).

DNA extraction, amplification, and sequencing

DNA was isolated from each sample using a standard phenol preparation (Hillis et al. 1990, DeSalle et al. 1993). We examined partial sequences of three ribosomal genes (two mitochondrial and one nuclear) and a partial sequence of cytochrome *b*. PCR products were prepared for DNA sequencing in several ways. In all cases the nuclear 18S rDNA fragments were GeneCleaned (BIO 101; Palumbi et al. 1991) and directly sequenced. PCR products of the mitochondrial genes (12S, 16S, and cytochrome *b*) were either GeneCleaned and directly sequenced or cloned into the TA vector (INVITROGEN) and sequenced (in such cases, at least two clones for each taxon were used to establish the sequence). We used the following primers: in the 18S gene region, 18sai0.7 (5′-ATTAAAGTTGTTGCGGTTT-3′) and 18sai0.79 (5′-TTAGAGTGCTYAAAGC-3′) (Wheeler et al. 1993), in the 12S gene region, 12SA (5′-GGTGGCATTTTATTTTATTAGAGG-3′) and 12SB (5′-CCGGTCTGAACTCAGATCACGT-3′) (Kocher et al. 1989, Hedges et al. 1993b), in the 16S gene region, 16SA (5′-CGCCTGTTTACCAAAAACAT-3′) and 16SB (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al. 1991), and in the cytochrome *b* region, H15149 (5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′) (Kocher et al. 1989) and L14724 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′) (Meyer et al. 1990). All sequencing was performed using the Sequenase system (U.S. Biochemicals) and double stranded templates. The sequences reported in this paper have been deposited in the EMBL Nucleotide Sequence Database (accession no. X95003–X95061).

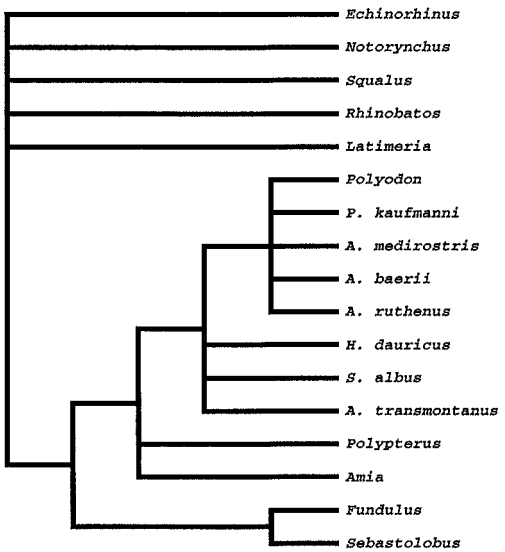

Figure 2. A phylogenetic tree for a combined (18sai0.7 plus 18sai0.79; 229 bp) region of fishes: eight acipenseriform species studied, *Polypterus senegalus*, *Amia calva*; four chondrichthyans, *Notorynchus cepedianus* (Hexanchiformes, Hexanchidae; Bernardi & Powers 1992), *Echinorhinus cookei* (Squalifomes, Squalidae; Bernardi & Powers 1992, Bernardi et al. 1992, M91179, GenBank), *Squalus acanthoides* (Squalifomes, Squalidae; Bernardi & Powers 1992, Bernardi et al. 1992, M91181, GenBank), and *Rhinobatos lentiginosus* (Stock & Whitt 1992, M97576, GenBank); *Latimeria chalumnae* (Stock et al. 1991b, L11288, GenBank); and two teleosts, *Fundulus heteroclitus* (Cyprinodontiformes, Fundulidae; Bernardi et al. 1992, M91180, GenBank), and *Sebastolobus altivelis* (Scorpaeniformes, Scorpaenidae; M91182, GenBank). *Squalus acanthias* and *Rhinobatos lentiginosus* were used as outgroups.

Outgroup choice

We chose *Polypterus senegalus*, a representative of a lineage often considered to be the sister group of Acipenseriformes plus Neopterygii (Patterson 1982), as our outgroup. Because the use of multiple outgroups is recommended in phylogenetic analyses (Watrous & Wheeler 1981), we also used *Amia calva* (Amiidae), generally regarded as the living sister species of teleosts (see Patterson 1973), as an outgroup. In the analysis of the partial sequence of the 18S gene we used two chondrichthyan species, *Squalus acanthias* and *Rhinobatos lentiginosus* (Bernardi et al. 1992, M91179; Stock & Whitt 1992, M97576), as outgroups.

DNA sequence alignment and phylogenetic analysis

We used equal weights for all nucleotide positions in all analyses. When multiple parsimonious trees were obtained for a particular analysis, successive weighting based on the retention index was used to choose among these multiple parsimonious trees (Carpenter 1988). DNA sequences for the mitochondrial 16S and 12S rRNA regions and the nuclear 18S rRNA regions were aligned using the program MALIGN (Wheeler & Gladstein 1993). Gap costs were varied in order to explore the effects of alignment parameters on phylogenetic inference (the results of varying alignment parameters on phylogenetic inference are discussed in detail in Fitch & Smith 1983, Gatesy et al. 1993, Hillis et al. 1994, Wheeler 1995, Wheeler et al. 1995). In most cases our alignments were extremely stable (i.e., alignment columns did not change by altering gap costs) and this stability suggests a low level of 'alignment ambiguity' (Gatesy et al. 1993). Consequently, the methods of 'culling' (Gatesy et al. 1993) and 'eliding' (Wheeler et al. 1995) were not applied to infer alignment. It was trivial to align cytochrome *b* sequences, which were also performed using MALIGN with a gap cost of 8. Parsimony trees for each of the four individual gene regions were generated separately using the PAUP 3.1 program (Swofford 1993) to examine the signal inherent in each gene region. Sequence alignments using a gap cost of 8 were arbitrarily chosen and were combined (Kluge 1989, Ernisse & Kluge 1993) into a single data matrix. Phylogenetic hypotheses were generated from this combined data matrix using PAUP. The degree of support for particular nodes in these trees was examined using the Bremer support index (Bremer 1988, Donoghue et al. 1993, Kallersjo et al. 1993).

Results and discussion

Gene regions

18S rDNA

The 18S rRNA gene is relatively slowly evolving in vertebrates and has been useful at higher taxonomic levels (e.g., Stock et al. 1991a). We used two se-

quencing primers in our analyses, 18sai0.7 and 18sai0.79. These primers are in a region of the 18S gene that varies in insects and other organisms (Wheeler et al. 1993). The 18sai0.7 sequences were invariant in all acipenseriform species investigated, whereas the 18sai0.79 fragment was variable at several positions. A low degree of 18S rRNA sequence divergence between *Scaphirhynchus* and *Polyodon* was reported previously (Stock et al. 1991a).

The phylogenetic tree for combined data sets for both fragments of the 18S gene for all fish species investigated so far is presented in Figure 2. Species used and origin of the sequences are explained in the legend to this figure. *Echinorhinus cookei* and *Rhinobatos lentiginosus* were chosen as outgroups. The tree statistics are shown in Table 6.

There is a high degree of similarity between the gene fragments under consideration in all four chondrichthyans and *Latimeria chalumnae*, and they differ from these fragments in actinopterigyans. There are two putative synapomorphies of acipenseriformes in the 18sai0.7 region: (1) all acipenserids and *Polyodon spathula* had an insertion of A between 684 and 685 comparatively to the homologous sequence of *L. chalumnae*, and (2) a T between positions 773 and 774 relative to *L. chalumnae*. Also, a change of A to T in position 771 (*L. chalumnae*) seems to be synapomorphic for all acipenseriform species. The most variable region of 18sai0.79 region in all groups of fishes examined so far lies between T and G in position 851 and 855 (*L. chalumnae*). In chondrichthyans and *L. chalumnae* there are three nucleotides, TCG, whereas in teleosts there are 7 nucleotides, TTCTCCT or TCTTTCT, and in species studied by us, only a part of the latter sequence, CCT.

16S mitochondrial rDNA

We obtained sequences for two parts of this gene: (1) a 147 nucleotide sequence using the 16SA primer (146 nucleotides for *Amia calva* and *Polypterus senegalus*), and (2) a 169 nucleotide sequence with the 16SB primer (164 nucleotides in *Amia calva*). Both regions were highly conserved, although there were differences in the 3′-part of the 16Sb fragment.

12S mitochondrial rDNA

Two overlapping short stretches of 12S mtrDNA, 12SA and 12SB, were sequenced. The final contiguous sequence consisted of 183 nucleotides in *Huso dauricus* and *Pseudoscaphirhynchus kaufmanni*, 184 nucleotides in *Polyodon spathula, A. medirostris, A. baerii, Scaphirhynchus albus*, and *Amia calva*, and 185 nucleotides in *Polypterus senegalus*. The 12S region is more variable than the 16S regions and yields several phylogenetically informative characters for examining higher level relationships (see below). Extremely low levels of variability, however, exist within the genera *Acipenser* and *Huso*.

Cytochrome b

The amplified region consisted of 270 base pairs, from the 7th to 97th codons according to Normark et al. (1991). Most variation occurs at the third posi-

Table 6. Tree statistics.

Gene	Number of characters	Apomorphies, total number	Number of phylogenetically informative characters	Number of trees	Number of steps	Consistency index	Retention index
(18sai0.7+ 18sai0.79) fragment	241	39	24	6	47	0.94	0.98
12S gene fragment	189	51	10	6	61	0.71	0.67
16S gene fragment	318	74	23	2	86	0.82	0.81
18S gene fragment	229	12	7	3	14	0.88	0.91
Cytochrome b gene fragment	270	163	56	3	187	0.62	0.54
Combined molecular characters	1006	300	96	1	354	0.65	0.59
Cytochrome *b* with additional species of *Acipenser*	270	163	56	2	216	0.54	0.52

tions of codons. For comparable lengths of the 16S and 12S sequences, the cytochrome *b* gene for the species examined by us has from three to four times more nucleotide changes.

Generic relationships within Acipenseriformes

Alignment and phylogenetic inference

First, we examined phylogenetic signal in the individual gene character sets by constructing separate cladograms for each of the four genes studied. Second, due to the small number of apomorphic characters for each gene, we used a combined approach (Miyamoto 1985, Kluge 1989, Ernisse & Kluge 1993) to infer phylogenetic relationships.

We used aggressive alignment parameters for the program MALIGN (build; treeswapping; random sequence addition; Wheeler & Gladstein 1993) in our computer searches. We avoided rearranging computer generated alignments based on eye judgment because of the arbitrary and inherently nonrepeatable nature of this approach and because the choice of gap:change cost ratios in DNA sequence alignment can greatly affect final alignment (Fitch & Smith 1983, Waterman et al. 1992, Gatesy et al. 1993). We performed several alignments with varying gap:change ratios, and found alignments for all three rRNA were very stable. There were few alignment ambiguities as judged by comparing alignments generated at gap:change costs of 2, 4, 8 or 16. The phylogenetic hypotheses generated by these various alignments were congruent, further supporting our notion that the alignments are very stable.

Individual gene trees

We report three consensus gene trees from sequences aligned using a gap:change ratio of 4 for the three rRNA genes (Figure 3). As noted above, cytochrome *b* sequences were aligned with a gap cost of 8 and the resulting gene tree is also shown in Figure 3. The tree statistics for each of the gene trees, including the number of apomorphies and phylogenetically important characters, is given in Table 6. The phylogenetically informative characters and

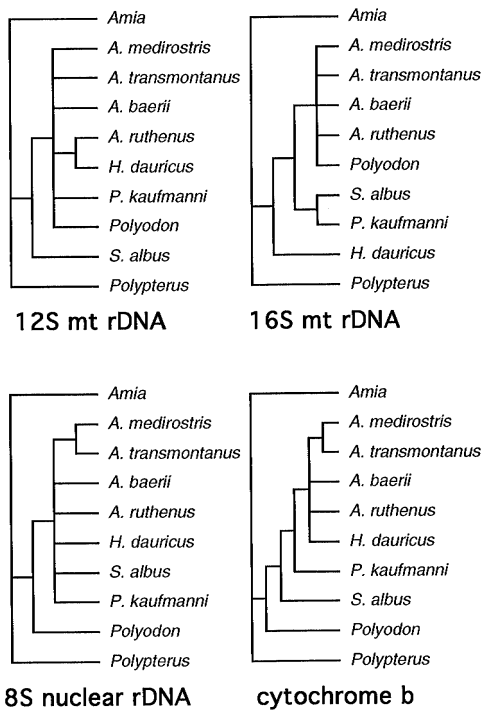

Figure 3. Consensus trees for individual gene regions examined for the 12S mtrDNA (189 bp), 16S mtrDNA (318 bp), 18S rDNA (229 bp), and cytochrome *b* genes (270 bp). *Amia calva* and *Polypterus senegalus* are outgroups. The tree statistics are in Table 6.

their positions in the sequences for each gene are shown in Figure 4.

In general, each gene tree alone showed low levels of resolution (Figure 3). Successive weighting of the individual character sets did not result in the choice of a single or fewer trees. Although character congruence was high as indicated by the relatively high consistency and retention indices, the number of informative characters for each character set was so low (Figure 4) that resolution of only a few nodes in each single gene tree was demonstrated.

One general observation, however, is that *Acipenser* was not found to be monophyletic in any of the four gene trees (Figure 3). For 12S mtDNA, *Huso dauricus* clustered with *A. ruthenus*, and no characters were found that hypothesized the remaining species of *Acipenser* as a group. For 16S mtDNA, *Polyodon spathula* clustered with the four species of *Acipenser* surveyed, and no character was found

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                          12S              16S                 18S

                     11111111         1111111122222233    11112
                   3644555667 3567789022234883999990 3422260
Node               5808567564 0033871068974699123670 3003553
-------------------------------- ---------------------- -------
A. medirostris     TCTAATGCAC ACGCTCACTAAGTTGAGTATGTA ATTTATT
A. transmontanus   TCTAATGCAC ACGCTGACTAAGTTGAGTATGTA ATTTATT
A. baerii          TCTAATGCAC ACGCTGACTAAGTTGAGTATGTA ATTTATC
A. ruthenus        TCTAATGC-T ACGCTGACTAACTTGAGTATOTA ATTTATC
Huso dauricus      TCTAATGC-T ACGCTGACCTAGTT-A-TATGTA ATTTACC
Scaph. albus       TCCGTGGCAC TCGCTGACCTAGTGGATGGTGTA ATTTACC
Pseudosc. kaufm.   TCCAATGC-C ACGCTGACCTAGTGGAGTGTGTA ATTTATC
Polyodon spathula  TCCAATGC-C ATGCTGAATAAGTTGAGGGTGAA ATATTTC
Amia calva         ATTAAGATAC CTATCAGCCTT-CT-GGTACTTG -CA-TCC
Polypterus seneg.  ATCGTAAT-G CTATCAGACTT-CT-G---CTAG -CA-TCC
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cytochrome b

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                                 1111111111111111111211122222222222222222
                    123334466667791112223344566677888889900111122444555566
Node                691019080346250147037281432585703679281703690207902358 14
-------------------------------------------------------------------------------
A. medirostris      GaCCCACTCAGTTATTGTCACTTTTGCCCTaCTCGGATATCACCCACACCCCGTCC
A. transmontanus    GaCCCATTCAGTTATTGTCACTCTTGCCCTGTTCGGATAcCACCCACACCCTGTCC
A. baerii           AGCCCACTCAOTTATCATCACCTTTGCCTTGCTCGAATGTTACCCACACCCCGTCC
A. ruthenus         AGCCCACTCAGTTAcCGTCACCTTCGCCCTaCTCGAATGTTACCCACACCCCGTCC
Huso dauricus       GGCCCACCCAGTTATTATCCTCTTTGCCTTGTCCGGACGcTCTCCACACCCCGcCC
Scaph. albus        AatCtACTCAGTTAcCACCGCCCCCCCCTTGCTCGCATAcCCCCCACGCCCTAcCC
Pseudosc. kaufm.    AatCtACTCAGTTAcCATtACCTTTGCCTTGCTCGAATGcTACtCACACCCCGctC
Polyodon spathula   TaCTCATCCAGTCAcTTCCACTCTTATCCCCCTCGACCATCACCTCCGTCCCAcAT
Amia calva          TatCGCTATCTACCACTCtCTATCCAATTCaTCTATCCAcCCTATCTGGTATTctC
Polypterus seneg.   GatTACCATCTATCACTTCACCTTCCATTATCTTACAAAcACCATCTATTACCTtT
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Figure 4. Phylogenetically informative characters (nucleotide composition at variable sites) for four gene regions studied. Numbers above the sequences refer to base pair position from the first base in the amplified fragments. The first position for the amplified fragment for 12S (No. 35) corresponds to No. 570 in the published 12S sequence for *Latimeria chalumnae* (Hedges et al. 1992), in 16S (No. 30) corresponds to No. 176 for *L. chalumnae* (Hedges et al. 1992), in 18S (No. 33) corresponds to No. 682 for *L. chalumnae* (Stock et al. 1991b), and in cytochrome *b* gene (No. 6) corresponds to No. 25 for *L. chalumnae* (Normark et al. 1991).

uniting *Acipenser* as monophyletic. In the 18S rDNA tree, there was again no evidence of monophyly of *Acipenser*. Finally, the cytochrome *b* tree grouped *A. medirostris*, *A. transmontanus*, *A. ruthenus* and *A. baerii* with *Huso dauricus*. *Acipenser medirostris* and *A. transmontanus* shared apomorphies in the 18S rDNA and cytochrome *b* trees, and such a grouping was not ruled out by either of the other two trees. *Pseudoscaphirhynchus* and *Scaphirhynchus* emerged as monophyletic in only one tree (16S, Figure 3).

Combined molecular tree and comparison to existing morphological hypothesis

The DNA sequence characters for the four gene regions were combined into a data matrix and each character was given equal weight. One parsimony tree was obtained using this combined molecular data matrix (Figure 5, Table 6).

Findeis (1993, 1997) used osteological characters for constructing a morphological phylogenetic hypothesis which focused on generic relationships among Acipenseriformes. Only a single parsimony tree resulted from his data set. He examined six species of acipenseriforms not included in our molecular survey (*Scaphirhynchus platorynchus*, *A. brevi-*

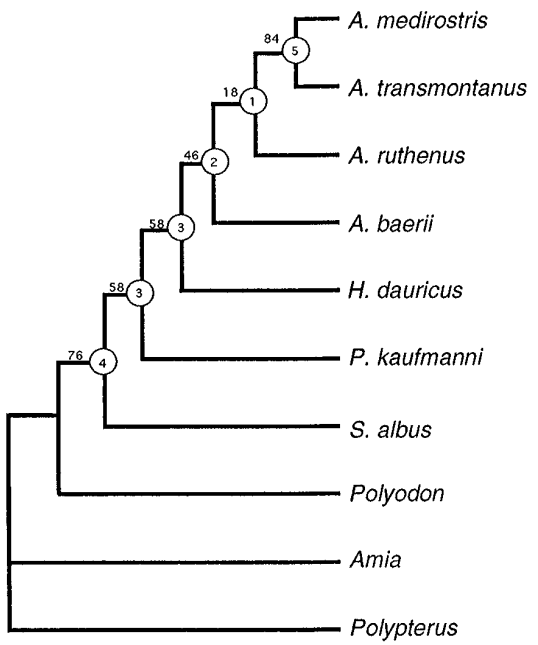

Figure 5. The single parsimony tree obtained for the combined set of molecular characters. *Amia calva* and *Polypterus senegalus* are outgroups. Decay indices (Bremer 1988, Donoghue et al. 1993) are shown at each node. Numbers above the branches represent bootstrap values computed by 1000 replications. The tree statistics are given in Table 6.

rostrum, *A. fulvescens*, *A. oxyrinchus*, and *Huso huso*). He did not examine the osteology of three species studied by us (*A. baerii*, *H. dauricus*, and *S. albus*). Thus, there is incomplete overlap of taxa surveyed by the two phylogenetic approaches, morphological and molecular. A further complication is that Findeis (1993, 1997) does not provide evidence that *Acipenser* is monophyletic, whereas our total molecular data set does (Figure 5).

Comparison of the osteological and molecular trees shows two major differences: (1) the placement of *Huso dauricus* and (2) the sister relationship of *Scaphirhynchus* and *Pseudoscaphirhynchus*. Findeis (1993, 1997) found that *Huso* is basal to the other Acipenseridae, and that the clade including all other sturgeons was well supported by osteological characters. Our combined molecular data, however, suggest that *Huso dauricus* is a sister-species to the genus *Acipenser* (Figure 5). Perhaps, this conflict between the molecular and morphological information results from the small number of molecular characters that are pertinent to the *Huso-Acipenser* sister group hypothesis. It is interesting, however, that many 19th century systematic studies placed *Huso* within *Acipenser* and that *Huso* was not elevated to a separate generic status until Brandt (1869). Later the generic status of *Huso* was still debated (for instance, Nikolukin 1970, Artyukhin 1995). Evidently, *Huso* warrants new attention from systematists.

The sister relationship of *Scaphirhynchus* and *Pseudoscaphirhynchus* is strongly supported by the morphological data (Findeis 1993, 1997), but it is not supported by our molecular characters. In Figure 5 *Scaphirhynchus* emerged as the sister taxon of all other sturgeons, and *Pseudoscaphirhynchus* emerged as the sister taxon of *Huso* and *Acipenser*. This is an interesting difference between the two phylogenies because conventional pre-cladistic ideas about relationships within Acipenseridae suggest that *Scaphirhynchus* plus *Pseudoscaphirhynchus* are basal members of the family (e.g., Zograf 1887). The fact that *Pseudoscaphirhynchus* and *Scaphirhynchus* did not group together is supported in our combined tree by relatively high decay indices (4 and 3 at the pertinent nodes in Figure 5) and bootstraps. We suspect that the traditional idea of this monophyly may be incorrect.

Relationships within the genus Acipenser

For this investigation we used partial sequences of the cytochrome *b* genes of eight Eurasian and four American species of the genus *Acipenser*. This data set includes 7 species absent in the previous molecular analysis because we did not sequence the ribosomal genes for these taxa. Taxa chosen represent all four species groups proposed by Artyukhin (1995, see above). The result of phylogenetic analysis is presented in Figure 6, and tree statistics, in Table 6.

Two parsimony trees were obtained. In the cytochrome *b* analysis, *Acipenser* is not monophyletic, and two main clades of species are seen in both trees. Two western American species, *A. medirostris* and *A. transmontanus*, group together and are a

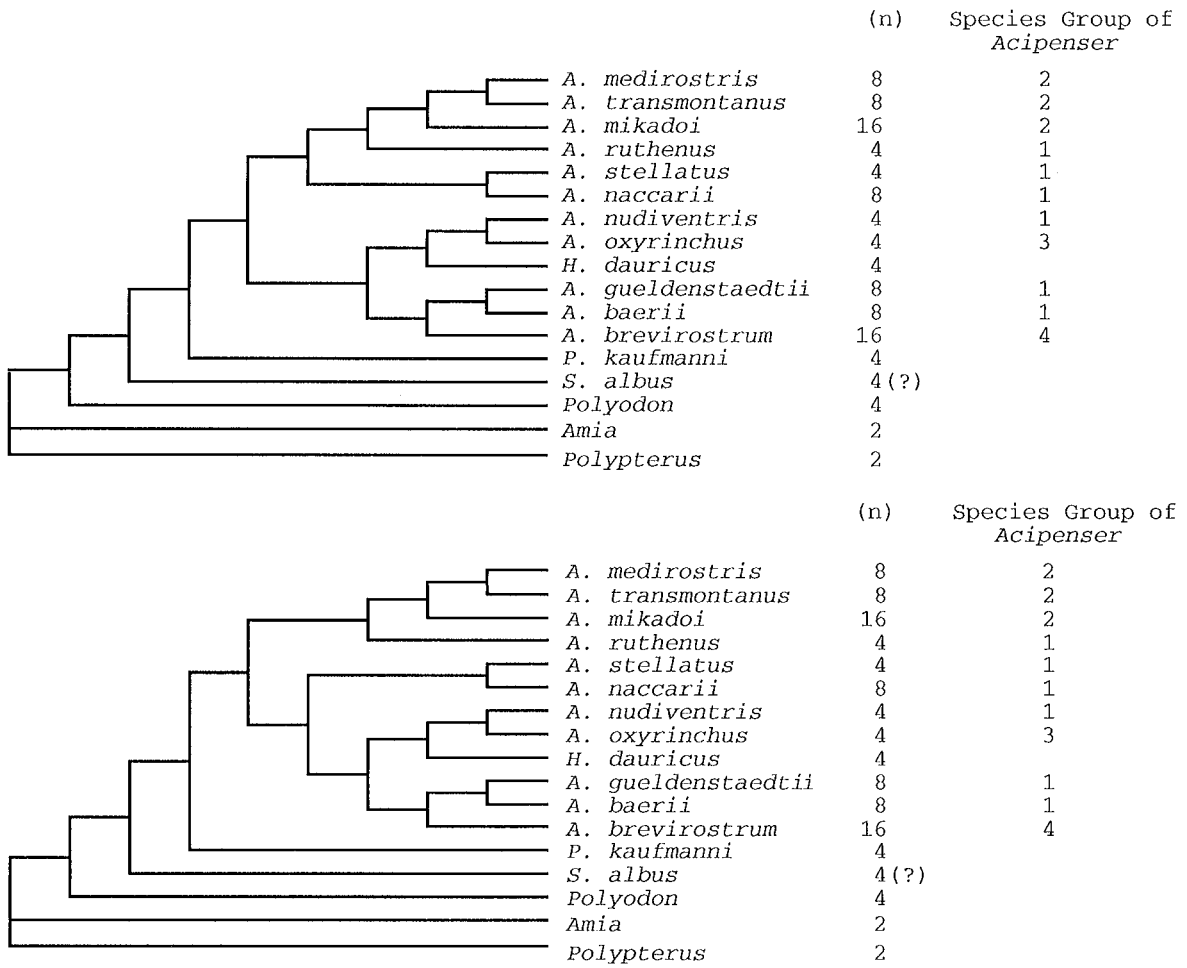

Figure 6. The two parsimony trees based on partial sequences (270 bp) of the cytochrome *b* gene regions from 11 species of *Acipenser* (representatives of all groups of species in Table 5), *Huso dauricus*, *Pseudoscaphirhynchus kaufmanni*, *Scaphirhynchus albus*, and *Polyodon spathula*. As in the previous analyses, *Amia calva* and *Polypterus senegalus* were used as outgroups. The ploidy of fishes and the group number for species of *Acipenser* are given to the right of species names. The tree statistics is shown in Table 6.

sister-group to *A. mikadoi*, whereas *A. ruthenus* is basal to all these species. Except for the freshwater *A. ruthenus*, all other species of the clade are typically anadromous sturgeons. The difference in the cytochrome *b* gene sequences between *A. mikadoi* and *A. medirostris* supports the previous assumption based on DNA ploidy that *A. mikadoi* is a separate species in spite of the fact that it is morphologically indistinguishable from *A. medirostris* (Birstein et al. 1993, Birstein 1994b). Unexpectedly, the ancestral form of this group seems to be related to *A. ruthenus*.

The second group is also unexpected. It consists of two clades. The first clade includes the European *A. nudiventris* grouped with the eastern American *A. oxyrinchus* (and *Huso dauricus*, see discussion above). Their sister-group consists of the European *A. gueldenstaedtii*, Siberian *A. baerii*, and an eastern American species *A. brevirostrum*. These relationships suggest that: (1) the Atlantic group of species is related to the Ponto-Caspian *A. nudiventris*; (2) a Ponto-Caspian European *A. gueldenstaedtii* is closely related to a Ponto-Caspian Siberian *A. baerii*; (3) both Ponto-Caspian *A. gueldenstaedtii* and *A. baerii* are related to the eastern North American

A. brevirostrum; and (4) both clades show transatlantic relationships for the species.

The position of a small clade consisting of *A. stellatus* and *A. naccarii*, is unresolved: in the first tree it is grouped with the first main clade, whereas in the second tree it clusters with the second main clade (Figure 6). Traditionally, the octoploid *A. naccarii* was considered to be closely related to the octoploid *A. gueldenstaedtii* (e. g., Tortonese 1989, Rossi et al. 1991, Artyukhin 1995), but not to the tetraploid *A. stellatus*.

According to the tree in Figure 6, ploidization occurred at least three times within the *Acipenser*: two octoploid ancestral forms were formed independently in *A. mikadoi-A. medirostris-A. transmontanus*, *A. gueldenstaedtii-A. baerii-A. brevirostrum*, and in *A. stellatus-A. naccarii*; two polyploidization events followed resulting in the appearance of *A. mikadoi* and *A. brevirostrum*. These data support our assumption (see above) that ploidization played a significant role in speciation within the *Acipenser*, but contradict a simple scheme of hypothetical relationships of the species of *Acipenser* published by Artyukhin (1995, see also Bemis et al. 1997). Except the close relatedness of *A. transmontanus* and *A. medirostris*, which is supported by other molecular data (Brown et al. 1996), the other relationships in Artyukhin's tree (Artyukhin 1995) are not supported by our molecular data.

We caution that the trees in Figure 6 are preliminary ones. Evidently, additional data should be obtained for better resolution of relationships among the species. We have already sequenced longer regions of the cytochrome *b* gene, as well as other genes (Birstein & DeSalle 1997). Our data do show, however, that phylogenetic relationships within the *Acipenser* can be reconstructed using even a partial sequence of the cytochrome *b* gene.

General lack of molecular variability among the genera of Acipenseriformes

We initially chose the gene regions described above because of the high degree of variability shown in other taxa of comparable divergence times (Meyer & Wilson 1990, Normark et al. 1991, Stock et al. 1991a, Hedges et al. 1993a). One surprising result of our study is the general lack of variability in the gene regions studied as compared to other animal groups, including teleosts (reviews in Meyer 1993, Meyer et al. 1993, Patarnello et al. 1994), some amphibians, most mammals and insects (for instance, Irwin et al. 1991, Hedges et al. 1993b, Wheeler et al. 1993). The slow rate of molecular evolution in acipenseriforms may be correlated with slow karyotypic evolution in these fishes (see above).

The other group of fishes with a low rate of evolution in 18S and mitochondrial genes is Chondrichthyes (Bernardi & Powers 1992, Martin et al. 1992, Martin & Palumbi 1993). Slow evolution of the 18S genes, as in acipenseriforms (see Figure 2), could be related to polyploidy (cryptoploidy) in these fishes (reviews in Schwartz & Maddock 1986, Birstein 1987, Stingo & Rocco 1991). But the nucleotide substitution rate in the cytochrome *b* gene in sharks is one sixth that of primates (Martin et al. 1992, Martin & Palumbi 1993), and this characteristic cannot be attributed to ploidy differences. Perhaps, it is caused by differences in the rate of accumulation of silent transversions (Martin & Palumbi 1993), but this remains a peculiar problem. It is interesting that the sequence of the region of the 18S gene under discussion in sharks is more similar to that in the coelacanth than to that in acipenseriforms (Figure 2).

It is evident that the molecular data (at least those presented here), as well as the cytogenetic data (see above), have restrictions in application to the phylogeny of Acipenseriformes at the generic level. Low levels of variability of the genes commonly used as phylogenetic tools (12S, 16S, 18S, and cytochrome *b*) suggests that some other, rapidly evolving gene regions such as the mitochondrial control region (D-loop, Shedlock et al. 1992) or larger portions of the cytochrome *b* or other mitochondrial structural genes (Normark et al. 1991) might be helpful for examining relationships among the genera of Acipenseridae.

In the meantime, our results suggest that the cytochrome *b* gene could be used for investigation of relationships among species of *Acipenser*. The cytochrome *b* data suggest that *Acipenser* is not monophyletic (due to the insertion of *Huso dauricus* into

this group), as considered before. We hope that future analyses involving longer regions of the cytochrome *b* gene and, possibly, some other protein coding genes will help to establish mono- or polyphyly of this genus.

Conclusions

(1) Little cytogenetic change has occured during the evolution of Acipenseriformes. Polyodontidae and Acipenseridae presumably originated from a tetraploid ancestor whose karyotype consisted of 120 macro- and microchromosomes with a DNA content about 3.2–3.8 pg per nucleus. Tetraploidization of the 60-chromosome ancestor possibly occurred at the early times of evolution of the Acipenseriformes, probably, during the origin of this group in the Mesozoic.

(2) No conclusions regarding interrelationships within Acipenseridae among *Huso*, *Acipenser*, *Scaphirhynchus*, and *Pseudoscaphirhynchus* can be made based on cytogenetic data. Divergence of these lineages of sturgeons occurred without polyploidization.

(3) Diversification within *Acipenser* was accompanied by appearence of octoploids (according to karyotypic and DNA content data) and 16n-ploids (according to DNA content data). The octoploid 240-chromosome sturgeon species have about 240 chromosomes and may have originated independently in different geographic areas. The two 16n-ploid species, *A. mikadoi* and *A. brevirostrum*, may be the youngest species within the genus.

(4) A study of partial sequences of genes from mitochondrial DNA (16S rDNA, 315 bp; 12S rDNA, 189 bp, and cytochrome *b*, 270 bp) and of one nuclear gene region (18S rDNA, 230 bp) demonstrated very low levels of variability in the eight acipenseriform species surveyed (*Polyodon spathula*, *Huso dauricus*, four species of *Acipenser*, *Scaphirhynchus albus*, and *Pseudoscaphirhynchus kaufmanni*). This low variability is unusual for these genes, which are commonly used as phylogenetic tools.

(5) The molecular tree based on combined data from all four genes had two major departures from the existing morphological hypothesis (Findeis, 1993, 1997): *Huso dauricus* was a sister-species to the genus *Acipenser* instead of being basal to all acipenseriforms, and *Scaphirhynchus* and *Pseudoscaphirhynchus* did not form a monophyletic group.

(6) A partial sequence of the cytochrome *b* gene (270 bp) was used to examine relationships within the genus *Acipenser*. Seven additional species of Acipenser were included in this part of the study (*A. brevirostrum*, *A. gueldenstaedtii*, *A. mikadoi*, *A. naccarii*, *A. nudiventris*, *A. oxyrinchus*, and *A. stellatus*). The data support the hypothesis that octoploid species appeared at least three times within the *Acipenser*. Also they show close relationships between the Eurasian *A. ruthenus* and the Pacific *A. mikadoi-A. medirostris-A. transmontanus*, between the European *A. gueldenstaedtii*, Siberian *A. baerii*, and American *A. brevirostrum*, between two European species, *A. stellatus* and *A. naccarii*, as well as a possible trans-Atlantic relationship between the Eurasian *A. nudiventris* and American *A. oxyrinchus* suggesting limited utility of geographic locality as an indicator of relationship.

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