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HOW MANY POLYPLOIDIZATION EVENTS TOOK PLACE IN ACIPENSERID EVOLUTION? NEW EVIDENCE FROM KARYOLOGICAL STUDY OF THE SAKHALIN STURGEON *Acipenser mikadoi* AND KALUGA *A. dauricus*

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Abstract

The hypothesis on diploid-polyploid relations in sturgeons was presented at first based on the karyological data demonstrated two groups of species with twice different chromosome sets (Nikoljukin 1972). The large amounts of recently accumulated diverse data (Fontana 1976, 2002, Vasil'ev et al. 1980, Vasil'ev 1985, 1999, 2009, Birstein & Vasil'ev 1987, Fontana et al. 2008a) prove polyploid evolution in Acipenseriforms in fact. The species with about 120 chromosomes have tetraploid origin, 250 – 270-chromosome species have octoploid origin, and the only found species with about 370 chromosomes, *A. brevirostrum* Lesueur, belongs to 12-ploid species (Table). But, since the 120-chromosome species practically reach the level of functional diploidization, two scales of ploidy levels in Acipenseriforms were presented (Vasil'ev 2009). 1. 'Evolutionary scale': diploid species (extinct now) – tetraploid species (120-chromosome) – octoploid species (250-chromosome) - 12-ploid species (370-chromosome). 2. 'Recent scale': diploid species (120-chromosome) – tetraploid species (250-chromosome) – hexaploid species (370-chromosome). The last scale is accepted for the discussion on polyploidization events in Acipenseriform evolution presented in this study.

Table. Chromosome numbers and ploidy levels in different acipenseriform species

Species	Chromosome number	Ploidy level		Reference
		'evolutionary scale'	'recent scale'	
<i>Polyodon spathula</i>	120	4	2	Dingerkus & Howell 1976
<i>Scaphirhynchus platyrhynchus</i>	~112	4	2	Ohno et al. 1969
<i>Acipenser sturio</i>	116±4	4	2	Fontana & Colombo 1974
<i>A. nudiventris</i>	118±2	4	2	Sokolov & Vasil'ev 1989
<i>A. ruthenus</i>	118±2	4	2	Fontana et al. 1975, Vasil'ev 1985
<i>A. stellatus</i>	118±2	4	2	Vasil'ev 1985
<i>A. oxyrinchus</i>	121±3	4	2	Fontana et al. 2008b
<i>A. huso</i>	116±4	4	2	Fontana & Colombo 1974
<i>A. gueldenstaedtii</i>	250±8	8	4	Vasil'ev 1985, Vlasenko et al. 1989
<i>A. persicus</i>	~258	8	4	Nowruzfashkhami et al. 2000
<i>A. baerii</i>	249±5	8	4	Vasil'ev et al. 1980
<i>A. naccarii</i>	239±7	8	4	Fontana & Colombo 1974
<i>A. brevirostrum</i>	~372	12	6	Kim et al. 2005
<i>A. transmontanus</i>	248±8	8	4	Fontana 1994
<i>A. sinensis</i>	264±4	8	4	Yu et al. 1987
<i>A. fulvescens</i>	262±6	8	4	Fontana et al. 2004
<i>A. mikadoi</i>	262±4	8	4	Vasil'ev et al. 2008, 2009
<i>A. medirostris</i>	249±8	8	4	Van Eenennaam et al. 1999
<i>A. schrenckii</i>	266±4	8	4	Our unpublished data
<i>A. dauricus</i>	268±4	8	4	Vasil'ev et al. 2008, 2009

The Sakhalin sturgeon *A. mikadoi* Hilgendorf belongs to very rare species and was not karyologically studied earlier. Its ploidy level was defined by indirect way based on DNA content value (Birstein et al. 1997). According to cited study DNA content in the Sakhalin sturgeon is very high: "in two times higher" (13.93 – 14.73 pg/nucleus) than in the octoploid sturgeons including the North American green sturgeon characterized by 8.82 pg/nucleus (Blackledge & Bidwell 1993). These data resulted in conclusion on the 16-ploid level and 500-chromosome karyotype in the Sakhalin sturgeon (Birstein et al. 1993). After aforementioned publication the Sakhalin sturgeon is treated as 500-chromosome species by several authors (Birstein & Bemis 1997a, b, Birstein et al. 1997, Birstein & DeSalle 1998, Ludwig et al. 2000, 2001, Birstein 2005). Moreover, Ludwig and co-authors (2001) concluded that *A. mikadoi* is octoploid species with about 500 chromosomes and classified *A. medirostris* Ayres as tetraploid species with about 250 chromosomes on the strength of their microsatellite analysis in spite of the absence of true grounds for these conclusions.

Young of the current year of the Sakhalin sturgeon used for karyological analysis in this study were obtained in the Anyui fishery factory (Khabarovsk region) in 2008 from the same female. 11 specimens with total body length (L_T) 81 - 95 mm were subjected to the injection by 0.4% colchicine solution with doses of 0.1 - 0.2 ml per fish. The karyotypes were studied in kidney cells and in cells from the head lymphoid organ (= meningeal myeloid tissue) by using methodology published earlier (Vasil'ev & Sokolov 1980). Most suitable metaphases were obtained in lymphoid organ cells due to their high mitotic activity. As a whole, 63 metaphases were analyzed.

The number of chromosomes in different metaphase plates of the Sakhalin sturgeon varied from 258 to 278 with 262 - 270 chromosomes occurred more often. Therefore the karyotype of this species includes 262 ± 4 chromosomes. The number of banded chromosome is 80 (Fig.) and the number of chromosome arms (NF) - 342 ± 4 . This data completely refutes previous misconceptions on ploidy level in this fish. Besides, newly obtained data on the karyotype of the kaluga *A. dauricus* Georgi proves the same ploidy level in this species (Vasil'ev et al., 2008, 2009) treated as undoubted 120-chromosome fish by most authors (Birstein et al. 1993, 1997, Birstein & DeSalle 1998, Fontana et al. 1999, 2001, 2008, Fontana 2002, Ludwig et al. 2001). These new karyological data enable to revise previous hypotheses on the number of polyploidization events in Acipenseriform fish evolution.

Ludwig et al. (2001) presumed four polyploidization events in Acipenserid evolution: the first of them has occurred in the ancestor of polyploid species from Atlantic group (the karyotype of *A. brevirostrum* Lesueur with about 372 chromosomes (Table) was described later); the second one has occurred in the ancestor of subclade included *A. schrenckii* Brandt, *A. transmontanus* Richardson and *A. sinensis* Gray; the third polyploidization event has occurred in the phylum included *A. mikadoi* and *A. medirostris* Ayres; and the fourth one resulted in the origin of *A. mikadoi* treated as 500-chromosome species. Thus, both this hypothesis and further data on the hexaploid set in *A. brevirostrum* presume five polyploidization events in Acipenserid evolution, namely two events in Atlantic sturgeon group and three ones in Pacific group. Birstein (2005) recognizes seven polyploidization events in phylogenetic Acipenserid lines: three events occurred within Atlantic phylum (the first of them occurred in the ancestor of *A. persicus* Borodin, *A. naccarii* Bonaparte, *A. baerii* Brandt, *A. gueldenstaedtii* Brandt et Ratzeburg, and *A. brevirostrum*, the second resulted in the origin of *A. brevirostrum*, and the third resulted in the origin of *A. fulvescens* Rafinesque) and four events occurred within Pacific phylum (the first one occurred in the ancestor of *A. transmontanus*, *A. schrenckii*, and *A. medirostris*, the second one resulted in the origin of *A. sinensis* Gray, and two successive events occurred in the phylum of *A. mikadoi*). In addition, Birstein (2005) considers that the kaluga *A. dauricus* Georgi belongs to 120-chromosome sturgeons and includes this species in Atlantic group. Whereas in further phylogenetic mtDNA gene analyses the kaluga is found clustered together with other Pacific sturgeons (Ludwig et al. 2001, Krieger et al. 2008, Mugue et al. 2008), and recent karyological analysis revealed 268 ± 4 chromosomes in this species (Vasil'ev et al. 2008, 2009). Thus, eight polyploidization events should be presumed after Birstein (2005) hypothesis and newly obtained karyological data.

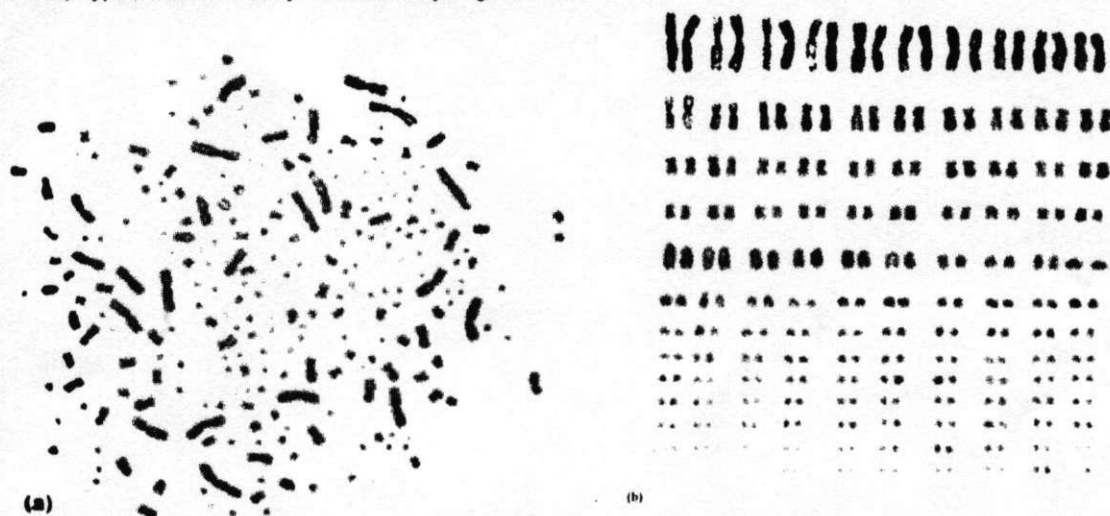


Fig. The metaphase (a) and karyotype (b) of the Sakhalin sturgeon *Acipenser mikadoi*; $4n=264$.

Recent data on the karyotype structure of the kaluga and the Sakhalin sturgeon (Vasil'ev et al. 2008, 2009) permit significant corrections in aforementioned interpretations of polyploid evolution in Acipenserid fish, peculiar in the Pacific species group. Since karyological analysis proves tetraploid origins of the kaluga and the Sakhalin sturgeons, as well as other Pacific group species, the only polyploidization event should be supposed for this group with different species originated from a common tetraploid ancestor. Two polyploidization events should be presumed in Atlantic group. The first one occurred in the common ancestor of Atlantic



tetraploid species originated by its further speciation, and the second one resulted in the origin of hexaploid *A. brevirostrum*.

Thus, at least three polyploidization events occurred in Acipenserid evolution. However, if polyploid species originated by hybridization with triploid forms existed at intermediate stages that seem most probable (Vasifov 1999, 2009), both triploid forms and final polyploids should possess genomes included haploid sets from different related diploid species. It means that polyploidization did not occur in different phylogenetic lineages, but resulted from the conjugation of phylogenetic lineages. In this case multiple polyploidization events should be presumed.

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HYBRIDIZATION BETWEEN PALLID AND SHOVELNOSE STURGEON

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Abstract

The pallid sturgeon, *Scaphirhynchus albus*, is listed as endangered by the United States Endangered Species Act. One of the listed potential threats to Pallid sturgeon is hybridization with the more common, shovelnose sturgeon, *S. platorhynchus*. Both species occur together in the Missouri River, the Mississippi River below the confluence with the Missouri, and in some major tributaries. Evidence suggesting hybridization occurs between these species has been observed at both morphological and genetic data. The goals of this study were to determine 1) where hybridization occurs throughout the pallid sturgeon's range, 2) if the hybridization is introgressive, and 3) to estimate the length of time hybridization has been occurring.

Fin clips from *Scaphirhynchus* specimens with morphologies consistent with pallid, shovelnose, and intermediate sturgeon were collected between 1999 and 2009 from each of the four recognized management units. The management units, listed from north to south, and upstream to downstream are the Great Plains Management Unit (GPMU, upper Missouri River), Central Lowlands Management Unit (CLMU, middle Missouri River), Interior Highlands Management Unit (IHMU, lower Missouri and middle Mississippi rivers), and Coastal Plains Management Unit (CPMU, lower Mississippi River). No sturgeon of known hatchery origin were included in these analyses.

Sixteen microsatellite loci were screened and morphological character index values were calculated. Bayesian model-based clustering was performed using the STRUCTURE software package to sort the genotypes into natural groups without a priori designation of species. We ran a single analysis using sturgeon from all locations pooled and separate analyses for each management unit. We used the Delta-K method to determine the most likely number of groups within *Scaphirhynchus*. Individuals were then assigned to groups by Q-value, which describes the proportion of an individual's genotype that originated in a particular group. Results from the Bayesian clustering were compared among geographic regions and were compared to morphological index scores. Simulations were performed to estimate genetic characteristics of F1 and first generation introgressed hybrids. Both genetic and morphological data were used to determine species as either pallid sturgeon, shovelnose sturgeon, or hybrid sturgeon. We compared results over geographic locations. Simulations were performed to estimate how Bayesian clustering would assign F1 and introgressed hybrids between pallid and shovelnose sturgeon.

Bayesian clustering of *Scaphirhynchus* sturgeon, in all cases, found two groups (Delta-K maximized at two). Thus, over all samples, and within each management unit, the results were consistent with the presence of genetically distinct pallid and shovelnose sturgeon. Results are reported with Q-values describing assignment to the pallid sturgeon genetic group. Individuals that are more pallid sturgeon-like are nearer to one, while more shovelnose-like samples are nearer zero. In all cases the results show evidence of pure pallid sturgeon, shovelnose sturgeon, and hybrid sturgeon. Structure results from the analysis (Figure 1) that pooled individuals from all management units clearly support the presence of pure pallid, pure shovelnose, and hybrid sturgeon.