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How Many Times Has Polyploidization Occurred During Acipenserid Evolution? New Data on the Karyotypes of Sturgeons (Acipenseridae, Actinopterygii) from the Russian Far East[1]

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Abstract—The karyology of species of sturgeon from the Russian Far East demonstrates that the karyotype of the Sakhalin sturgeon (*Acipenser mikadoi*) includes 262 ± 4 chromosomes with 80 biarmed chromosomes and the number of chromosome arms (NF) 342 ± 4, the karyotype of the Amur sturgeon (*A. schrenckii*) includes 266 ± 4 chromosomes with 92 biarmed chromosomes and NF 358 ± 4, and the karyotype of the kaluga (*A. dauricus*) consists of 268 ± 4 chromosomes with 100 biarmed chromosomes and NF 368 ± 4. These results prove that all western Pacific sturgeon species are from a tetraploid origin, based on a recent ploidy scale. This suggests that at least three polyploidization events have occurred during the evolution of Acipenseridae. However, if polyploid species originated by hybridization between diploid species, there may have been more polyploidization events in this group of fishes.

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A large amount of recently accumulated data from diverse sources confirms that polyploid evolution has occurred in acipenseriform fishes. At the same time, the problems of ploidy levels in different species, as well as the ways in which polyploidization has taken place, continue to be unresolved (Vasil'ev, 2009), even though most phylogenetic investigations use or interpret data on ploidy levels within Acipenseriformes (Vasil'ev et al., 1980; Vasil'ev, 1985; Birstein and Vasil'ev, 1987; Birstein et al., 1997; Birstein and DeSalle, 1998; Ludwig et al., 2000, 2001; Fontana, 2002; Krieger et al., 2008). The karyotype of several sturgeon species has remained unstudied, further limiting the understanding the evolution of polyploidy in these fishes. Further, the ploidy levels in several species were defined through indirect methods and require verification. For example, for the karyology of the Sakhalin sturgeon, *Acipenser mikadoi* Hilgendorf, which is a very rare species, has not been studied directly. Instead its ploidy level was defined by its DNA content, which was determined by flow cytometry (Birstein et al., 1993). According to this value, the DNA content in the Sakhalin sturgeon is very high (13.93–14.73 pg/nucleus). The DNA content for this species was estimated to be "two times higher" (Birstein et al., 1993) than in octoploid sturgeons, including the closly related North American green sturgeon (*A. medirostris* Ayres), which is characterized by 8.82 pg/nucleus (Blacklidge and Bidwell, 1993). The Sakhalin sturgeon was therefore concluded to have a 16-ploid level and 500-chromosome karyotype (Birstein et al., 1993); this assumption was accepted by several authors (Birstein and Bemis, 1997; Birstein et al., 1997; Birstein and DeSalle, 1998; Ludwig et al., 2000, 2001; Birstein, 2005).

Another case in which the karyotype requires verification is the kaluga, *A. dauricus* Georgi. (In several recent studies the *Huso*, previously used for this species, was found to be polyphyletic based on genetic and karyological evidence [Ludwig et al., 2000, 2001; Robles et al., 2004; Krieger et al., 2008; Vasil'ev et al., 2008, 2009], as well as the absence of any morphological characters to distinguish it and the great sturgeon *A. huso* Linnaeus from all other species of sturgeons [Vasil'eva et al., 2009a]). The karyotype of the kaluga was first studied through imperfect methods, which resulted in an erroneous value of 60 chromosomes (Burtzev et al., 1973). The 60-chromosome karyotype was later formally transformed to a 120-chromosome karyotype (Burtzev et al., 1976) in conformity with the published karyotype of the shovelnose sturgeon,

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Scaphirhynchus platorynchus (Rafinesque), which is characterized by 112 ± 5 chromosomes (Ohno et al., 1969). Thus, the kaluga was treated as a 120-chromosome species by most authors (Birstein et al., 1993, 1997; Birstein and DeSalle 1998; Fontana et al., 1999, 2001, 2008a; Ludwig et al., 2001; Fontana, 2002; Artjukhin, 2008).

Misconceptions on the ploidy level of the Sakhalin sturgeon and kaluga have been refuted by recent karyological studies of these species (Vasil'ev et al., 2008, 2009; Vasil'eva et al., 2009b). The purpose of this study is to comment further on the ploidy levels of these two species, as well as to present newly obtained data on the karyotype of the Amur sturgeon *A. schrenckii* Brandt, which had previously been described (Song et al., 1997). This study also revises previous hypotheses regarding the number of polyploidization events in the evolution of acipenseriform fishes based on mentioned newly obtained karyological data.

MATERIAL AND METHODS

Young of the year (YOY) kaluga, Sakhalin and Amur sturgeons that were used for karyological analysis were obtained in the Anyui Fishery Factory (Khabarovsk Region) in 2008. The offspring of the Sakhalin sturgeon were from the same female, whereas the offspring of both the Amur sturgeon and kaluga represent progenies from different females. In addition, one-year old individuals of Amur sturgeon that were reared in this institution were also used. In total, 11 YOY Sakhalin sturgeon (81–95 mm total body length, TL), 12 YOY Amur sturgeon (57–82 mm TL), 11 YOY kaluga (45–120 mm TL), and four yearlings of the Amur sturgeon (245–305 mm TL) were injected with 0.4% colchicine solution (0.1–0.2 ml per fish for small individuals and about 0.75 ml for one-year old specimens). Ten YOY Amur sturgeons were very small and died soon after injection, whereas other fishes successfully survived and after three hours were used for obtaining tissues for slide preparation.

The karyotypes were studied in kidney cells and in cells from the head lymphoid organ (Gurtovoi et al, 1976), which is also known as meningeal myeloid tissue (Fänge, 1986) or the "chondral brain" (Suvorov, 1948). The lymphoid organ is composed of reddish or reddish-brown tissue positioned within the cranial cavity in a saddle like manner above the posterior part of the brain and the anterior part of the spinal cord. It occurs in chondrosteans and holosteans and belongs to hemopoietic tissue (Ivanova, 1983; Fänge, 1986). The method for karyological study in sturgeons using cells from the lymphoid organ follows Vasil'ev and Sokolov (1980). Most suitable metaphases were obtained in lymphoid organ cells due to their high mitotic activity. In total 58 metaphase plates from 11 specimens of Sakhalin sturgeon, 63 metaphase from 11 specimens of kaluga, and 43 metaphases from six specimens of Amur sturgeon were analyzed.

Fig. 1. The metaphase (a) and karyotype (b) of the Sakhalin sturgeon *Acipenser mikadoi*; $2n = 264$.

RESULTS

The number of chromosomes in different metaphase plates varied from 258 to 278 (most between 262–270) in the Sakhalin sturgeon, from 258 to 278 (most between 262–270) in the kaluga, and from 253 to 272 (most between 262–270) in the Amur sturgeons. Therefore the karyotypes of these species include 262 ± 4, 268 ± 4, and 266 ± 4 chromosomes. More precise calculation of chromosome numbers in acipenserids is difficult, because these fishes have a great number of micro-chromosomes. The number of biarmed chromosome in the Sakhalin sturgeon is 80 (Fig. 1) and the number of chromosome arms (NF)—342 ± 4; in the kaluga the number of biarmed chromo-

Fig. 2. The metaphase (a) and karyotype (b) of the kaluga sturgeon *Acipenser dauricus*; $2n = 270$.

some is 100 (Fig. 2), NF 368 ± 4, and in the Amur sturgeon—92 (Fig. 3) and 358 ± 4.

DISCUSSION

Ploidy Levels of Sturgeons from the Far East of Russia

The karyological data presented here for three sturgeon species from the Far East of Russia show them to have about 250 chromosomes in their karyotypes. The karyotype with 266 ± 4 chromosomes obtained for the Amur sturgeon is similar with one previously described for this species by Song et al. (1997), who found this species to have 238 ± 8 chromosomes (Table). A more detailed comparative analysis of these data is impossible because the karyotype structure presented by Song et al. (1997, Fig. 2) is unsuitable for such analysis, and in fact it is inconsistent with the metaphase plate illustrated in their paper (Song et al., 1997, Fig. 1).

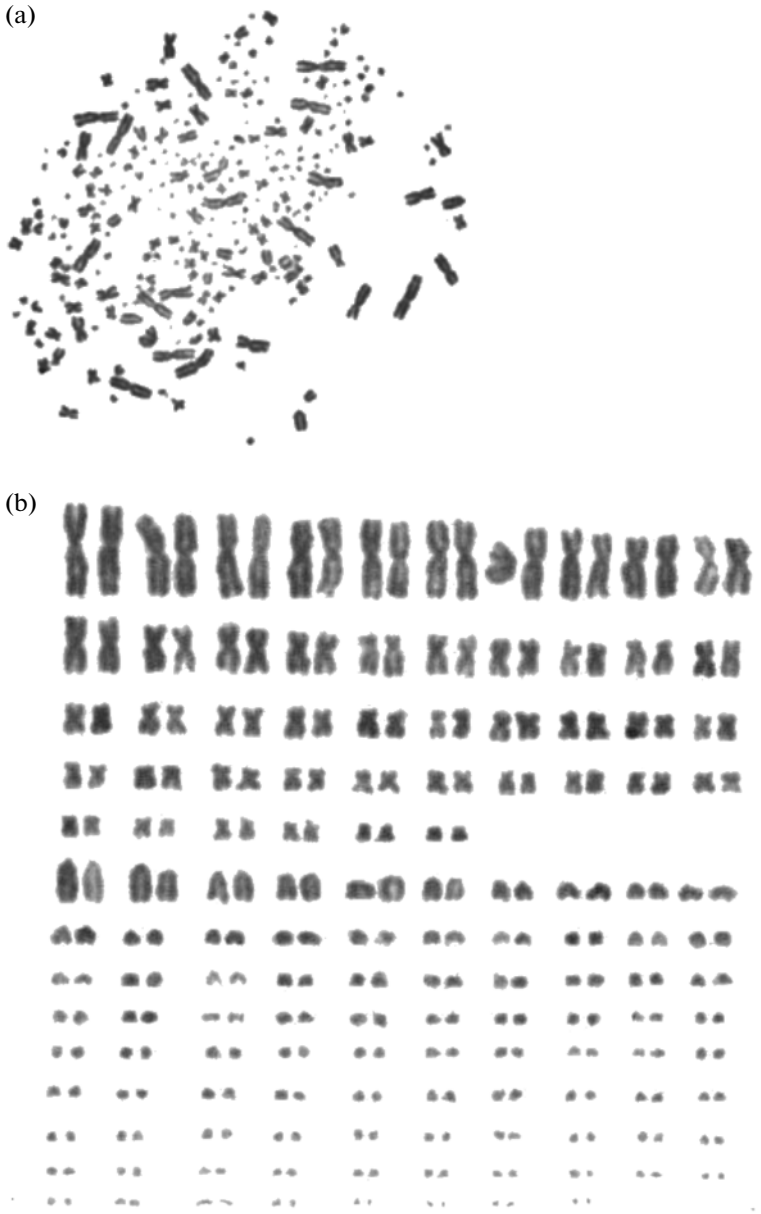

Fig. 3. The metaphase (a) and karyotype (b) of the Amur sturgeon *Acipenser schrenckii*; $2n = 268$.

A 250 chromosome ploidy level in the Sakhalin sturgeon is confirmed by the work of Vishnyakova et al. (2008), who found 247 ± 33 chromosomes in this species by using cell culturing (Vishnyakova et al., 2008). Moreover, a 250-chromosome karyotype of the Sakhalin sturgeon was concluded from the unpublished study of hybrids between this species and the Siberian sturgeon (*Acipenser baerii* Brandt) by V. Arefjev (Artjukhin, 2008; Vasil'ev, 2009).

In contrast, Ludwig et al. (2001) concluded that *A. mikadoi* is an octoploid species with about 500 chromosomes and classified the closely related species *A. medirostris* as tetraploid with about 250 chromosomes based on their microsatellite analysis. These conclusions look very questionable, because the microsatellite analysis demonstrated eightsomic allelic band pattern both in *A. mikadoi* and *A. medirostris* at two studied loci (Afu-57, Afu-68) and tetrasomic or disomic patterns for the rest three loci (Ludwig et al., 2001). Thus, it seems that aforementioned interpretation is caused by the assumption about ploidy relation between these sturgeons species accepted earlier. But additionally, the differences in DNA content between the Sakhalin sturgeon and North American green sturgeon were confirmed in further studies of these species. Flow cytometric histograms for four Sakhalin sturgeons caught along the coast of Hokkaido and 30 cultured specimens of the Amur sturgeon demon-

strated that the mean DNA content of *A. mikadoi* is about 1.14 times higher than the mean value of *A. schrenckii* (Omoto et al., 2004). Because the DNA content for *A. schrenskii* obtained by flow cytometry varies from 11.59 to 11.73 pg (Yin et al., 2004), these data confirm that the DNA content of the Sakhalin sturgeon is significantly higher than that of most 250-chromosome species.

Similarly, our data on the karyotype of the kaluga contradict the ploidy level previously calculated for this species by using indirect methods. The nuclear DNA content measured by flow cytometry in the kaluga by Birstein et al. (1993) corresponds to the 120-chromosome condition that had been attributed to this species. Additionally, these authors obtained values of 3.74–3.81 pg for two specimens of the kaluga and 8.24–8.42 pg for two specimens of the bester, an artificial hybrid of *Acipenser huso* and *A. ruthenus* Linnaeus that has about 118 chromosomes (Arefjev, 1989).

Therefore, taking into consideration the 268 ± 4-chromosome karyotype found by us for the kaluga, we suspect that these samples may have been mixed up by Birstein et al. (1993). This confusion is probable as the phylogenetic analysis conducted by Birstein and DeSalle (1998) recovered their "kaluga" in a group with *A. huso* and *A. ruthenus*, as would be expected for the bester. In subsequent phylogenetic mtDNA analyses the kaluga has been found to be related to other Pacific sturgeons (*A. medirostris, A. mikadoi, A. schrenckii, A. transmontanus* Richardson, *A. sinensis* Gray, *A. dabryanus* Duméril), while *A. huso* is included in an Atlantic group of sturgeons and represents the sister taxon of either diploid and tetraploid subgroups (Ludwig et al., 2001; Krieger et al., 2008; Mugue et al., 2008) or for the group including the Ponto-Caspian sturgeons and *A. baerii* Brandt (Fain et al., 2001).

A similar low value (4.77 pg) of the nuclear DNA content was obtained for the kaluga by Yin et al. (2004), which may have been caused by methodological and/or technical errors, as we did not find any difference in the size of cells from the head lymphoid organ between kaluga and both the Sakhalin and Amur sturgeons, which have DNA content values of 13.93–14.73 and 11.59–11.73 pg, respectively (Birstein et al., 1993; Yin et al., 2004). If the low value of the nuclear DNA content in kaluga is true, the ratio of its cell size and those from both Sakhalin and Amur sturgeons should be at least 1:2. Another possibility is the existence of two "forms" of kaluga with different ploidy levels, as it was hypothesized for the Amur sturgeon after a comparative analysis of the DNA content values (Zhang et al., 1999). Some authors distinguish two forms of the kaluga: the first a semi-diadromous population inhabiting the Gulf of Amur and the second a non-migratory freshwater form (Berg, 1948; Sokolov, 1989; Krykhtin and Svirskii, 1997). A semi-diadromous kaluga is presumed to be represented by winter and spring races (Berg, 1948). Different morphs, called brown and grey (Krykhtin and Svirskii, 1997) or short-snouted and long-snouted (Novomodny et al., 2004), are defined by some other authors for the Amur sturgeon, but their morphological, genetic and ecological features have not yet been specifically investigated. However, there are no grounds to connect these ecological forms with the observed discrepancy between values of the DNA content obtained by different methods and the true ploidy in sturgeon species. Moreover, recent observations suggest that most kaluga individuals have an early, extended anadromous life history phase, with adults from entirely freshwater stocks absent in the Amur River (Shmigirilov et al., 2006).

Ludwig et al. (2001) classified the kaluga as functional diploid together with 120-chromosome sturgeon species. These authors employ a recent scale for ploidy levels in Acipenseriformes. This scale presumes diploid-tetraploid-hexaploid relations, with a diploid karyotype including 120 chromosomes (due to its significant functional diploidization). In contrast an evolutionary scale presumes diploid-tetraploid-octoploid-12-ploid relationships, with 120 chromosomes in tetraploid set (Vasil'ev, 2009; table). However, the microsatellite analysis demonstrates disomic allelic band patterns in *A. dauricus* at four of the five loci that were studied. At one locus, Afu-68, tetrasomic pattern was revealed (Ludwig et al., 2001), whereas 120-chromosome species have disomic patterns at every loci examined. The number of microsatellite loci with known disomic inheritability approaches 36% in functional tetraploid sturgeons (Welsh et al., 2003). Therefore, the predominance of loci with disomic patterns among five studied loci in kaluga should be regarded as a chance phenomenon, but not evidence of its diploid state. Moreover, only three kaluga specimens were studied, while the samples of true 120-chromosome species included from 15 (*A. nudiventris* Lovetsky) to 150 (*A. ruthenus*) specimens (Ludwig et al., 2001). Therefore, the allelic variability in the kaluga seems to be understated for the loci that were studied. Nevertheless, it should be stressed that both of the discussed methods (microsatellite analysis and DNA content value) are indirect ones, and the karyological study is the only way to define ploidy level of any organism.

Moreover, the first information about our new results on karyotypes of the Sakhalin sturgeon and kaluga (Vasil'ev et al., 2008) appeared at the site "The chromosomes of Acipenseriformes" (http://web.unife.it/progetti/geneweb/doku.php?id=start) by Prof. Francesco Fontana (University of Ferrara, Italy) certainly stimulated further studies on polyploidy in sturgeons. As a result, Japanese scientific team presented re-evaluation of DNA content in several sturgeon species at the 6th International Symposium on Sturgeon (Wuhan, China, October 2009). These authors cite the karyotype of *A. mikadoi* from aforementioned site (264 chromosomes) and present their new data on DNA content in this species (Zhou

Chromosome numbers and ploidy levels in different acipenseriform species

Species	Chromosome number	Ploidy level		Reference
		"evolutionary scale"	"recent scale"	
Polyodon spathula (Walbaum)	120	4	2	Dingerkus and Howell, 1976
Scaphirhynchus platorynchus (Rafinesque)	~112	4	2	Ohno et al., 1969
Acipenser sturio Linnaeus	116 ± 4	4	2	Fontana and Colombo, 1974
A. nudiventris Lovetsky	118 ± 2	4	2	Arefjev, 1983; Sokolov and Vasil'ev, 1989a
A. ruthenus Linnaeus	118 ± 2	4	2	Fontana et al., 1975; Vasil'ev, 1985; Birstein and Vasil'ev, 1987
	118 ± 4	4	2	Ráb, 1986; Fontana, 1994
A. stellatus Pallas	118 ± 2	4	2	Vasil'ev, 1985; Birstein and Vasil'ev, 1987
A. oxyrinchus Mitchill	121 ± 3	4	2	Fontana et al., 2008b
A. huso Linnaeus	116 ± 4	4	2	Fontana and Colombo, 1974; Vasil'ev, 1985
A. gueldenstaedtii Brandt and Ratzeburg	250 ± 8	8	4	Vasil'ev, 1985; Vlasenko et al., 1989
A. persicus Borodin	~258	8	4	Nowruzfashkhami et al., 2000
A. baerii Brandt	249 ± 5	8	4	Vasil'ev et al., 1980; Sokolov and Vasil'ev, 1989b
A. naccarii Bonaparte	239 ± 7	8	4	Fontana and Colombo, 1974
A. brevirostrum Lesueur	~372	12	6	Kim et al., 2005
	372 ± 6	12	6	Fontana et al., 2008a
A. transmontanus Richardson	248 ± 8	8	4	Fontana, 1994
	~271	8	4	Van Eenennaam et al., 1998
A. sinensis Gray	264 ± 4	8	4	Yu et al., 1987
A. fulvescens Rafinesque	262 ± 6	8	4	Fonatana et al., 2004
A. mikadoi Hilgendorf	262 ± 4	8	4	Vasil'ev et al., 2008, 2009; this study
A. medirostris Ayres	249 ± 8	8	4	Van Eenennaam et al., 1999
A. schrenskii Brandt	238 ± 8	8	4	Song et al., 1997
	266 ± 4	8	4	This study
A. dauricus Georgi	268 ± 4	8	4	Vasil'ev et al., 2008, 2009; this study

et al., 2009a) as well as some other sturgeons including kaluga (Zhou et al., 2009b). Their study on DNA content by flow cytometry in adult *A. mikadoi* (6 specimens) and *A. dauricus* (4 specimens) collected off Hokkaido, Japan revealed values of 8.0–9.1 pg/N and 8.3–8.4 pg/N, accordingly. These values significantly differ from ones previously reported for both species but very close to those obtained for other 250-chromosome sturgeon species, namely *A. schrenckii* (7.9–8.2 pg), *A. transmontanus* (8.5–9.0), *A. baerii* (7.8–8.0), *A. fulvescens* Rafinesque (7.9–8.0), and *A. gueldenstaedtii* Brandt and Ratzeburg ("4.2/8.0"), or previously reported for *A. medirostris*. Thus, newly obtained data on DNA content entirely confirm our results on about 250-chromosome karyotype in the Sakhalin sturgeon and kaluga. However, the DNA content study on artificially propagated 19 days-old pure species larvae and hybrid larvae from crosses of different sturgeon species revealed "intra-specific polyploidy" in some progenies. The progeny of kaluga included several triploids with DNA content 12.3–13.1 pg/N, whereas in the Sakhalin sturgeon, high frequencies of triploid larvae (13.0–14.0 pg/N) as well as a few tetraploid larvae (17 pg/N) were detected (Zhou et al., 2009b). For "triploid" *A. mikadoi* larvae with 16–17 pg/N these authors presented the karyotype with 360–390 chromosomes (Zhou et al., 2009a). In spite of such discrepancy between ploidy interpretation, DNA content and karyotype, the presence of specimens with different ploidy in the same progeny is evident. And the authors concluded that their results suggested "the possible existence of polyploid mikado sturgeon indi-

viduals in nature" (Zhou et al., 2009a, p. 215) and "intra-specific genome size variation still occurs in sturgeon species at present" (Zhou et al., 2009b, p. 62). Such concept allows to escape the necessary criticism of former erroneous hypotheses on ploidy levels in the Sakhalin sturgeon and kaluga, as well as aforementioned interpretation of microsatellite data.

However, intra-specific variability in DNA content was demonstrated in artificially produced progenies from different pares of sturgeons with "normal" ploidy levels. Besides pure larvae of the Sakhalin sturgeon and kaluga, "polyploid" specimens also occurred among hybrids between these species and those between the Sakhalin sturgeon and the bester, among pure progenies of bester, *A. ruthenus, A. fulvescens*, and *A. transmontanus* (Zhou et al., 2009b). But it does not mean that any sturgeon species normally is represented by the mixture of specimens with different ploidy levels, although a few polyploids may occur in nature. The appearance of specimens with atypical ploidy level is more probable in artificially produced progenies as a result of two possible mechanisms. Firstly, polyploid specimens may originate from dispermic fertilization of egg. Two features of sturgeon eggs make dispermic fertilization possible: (1) multiple micropyles allow several spermatozoa to penetrate the egg and (2) there are no intracellular mechanisms to exclude super-numerous spermatozoa from development (Ginsburg, 1968). For example, the method of dispermic androgenesis is successfully used in different sturgeon species (Grunina et al., 1995, 2009). In this method weakly diluted (1:10 instead of the commonly used 1:100) sperm is used to cause polyspermic fertilization (see Grunina et al., 2009). The fertilization of unreduced diploid egg by single spermium can be other reason of occurrence of polyploid individuals in progeny. Such unreduced eggs more often occur in over-matured gonads of females rearing in artificial conditions. Thus, the appearance of specimens with atypical DNA content in 19-days-old larvae of different sturgeon species (Zhou et al., 2009a, b) should be interpreted as a result of engaging of any of two mentioned mechanisms (or both of them in a few cases of tetraploids) by disturbance of normal breeding conditions. Of course, similar situations may occur in nature, but not so often to convert species into diploid-polyploid mixture.

In summary, according to recent data the discussion on ploidy level in the Sakhalin sturgeon and kaluga should be considered of historical interest now. Indeed, the species of sturgeon from the Far East of Russia are tetraploid according to recent ploidy scale, or are octoploid according to an evolutionary scale. Consequently all Pacific species have the same ploidy and are characterized by chromosome numbers varying between about 260–270, except for *A. medirostris* which has about 249 chromosomes. In contrast sturgeon species of the same ploidy level from the Atlantic group generally have fewer than 260 chromosomes (Table).

The Number of Polyploidization Events in Acipenserid Evolution

The first hypothesis concerning diploid-polyploid relations in sturgeons was based on karyological data and concluded that there were two groups of species with different chromosome sets (Nikoljukin, 1972; Burtzev et al., 1973). The evolution of polyploidy in acipenseriform fishes subsequently has been analyzed by a number of authors (e.g., Vasil'ev et al., 1980; Vasil'ev, 1985, 1999, 2009; Birstein and Vasil'ev, 1987; Fontana, 2002; Fontana et al., 2008a).

Ludwig et al. (2001) concludes that four polyploidization events occurred in acipenserid evolution: (1) in the common ancestor of polyploid species from the Atlantic group (the karyotype of *A. brevirostrum* was described after Ludwig et al.'s 2001 study; table); (2) in the ancestor of subclade including *A. schrenckii, A. transmontanus*, and *A. sinensis* Gray; (3) in the group including *A. mikadoi* and *A. medirostris;* and (4) in the origin of *A. mikadoi* (which was believed to be 500-chromosome species). However, following from the data on the hexaploid *A. brevirostrum* (table), there would be five polyploidization events in acipenserid evolution in this scenario, corresponding to two events in the Atlantic sturgeon group and three in the Pacific group.

Birstein (2005) recognizes seven polypoidization events within Acipenseridae: three within the Atlantic group (1, in the common ancestor of *A. persicus* Borodin, *A. naccarii* Bonaparte, *A. baerii, A. gueldenstaedtii* Brandt and Ratzeburg, and *A. brevirostrum*, 2, in *A. brevirostrum* and 3, in *A. fulvescens* Rafinesque) and four in the Pacific group (1, in the common ancestor of *A. transmontanus, A. schrenckii*, and *A. medirostris*, 2, in *A. sinensis*, and 3, and 4, two successive events in *A. mikadoi*). However, Birstein (2005) records kaluga as a 120-chromosome sturgeon and includes this species in the Atlantic sturgeon group. But according to our data kaluga belongs to about 260-chromosome species. Additionally, this species has been found to be within the Pacific sturgeon group in other phylogenetic hypotheses generated by mtDNA gene sequences (Ludwig et al., 2001; Krieger et al., 2008; Mugue et al., 2008). Therefore, eight polyploidization events should be assumed in conformity with Birstein' (2005) hypothesis and recent data.

The data presented on the karyotypes of the Sakhalin sturgeon and kaluga (Vasil'ev et al., 2008, 2009, this study) contributes significant corrections for the interpretation of polyploid evolution in Acipenseridae, especially in the Pacific species group. Tetraploid origins of both species have been proved by karyological data, and, following from the tetraploid state of all other Pacific sturgeons (table), we conclude that only a single polyploidization event occurred in the com-

mon ancestor of the Pacific species group. In contrast, two polyploidization events likely occurred in the Atlantic species group: (1) in the common ancestor of tetraploid Atlantic species and (2) in the origin of hexaploid *A. brevirostrum*. Thus, at least three polyploidization events occurred in the evolution of the family Acipenseridae. However, if polyploid species originated by hybridization with triploid forms at intermediate stages, which seems probable (Vasil'ev, 1999, 2009), both triploid forms and the final polyploids should possess genomes consisting of haploid sets from different related diploid species. This would mean that polyploidization did not occur in different phylogenetic lineages, but rather resulted from the conjugation of phylogenetic lineages. If this occurred multiple polyploidization events should be presumed.

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