Cytological Analysis of the Reciprocal Hybrids between Low- and High-Chromosome Acipenserids, the Great Sturgeon, *Huso huso* (L.), and the Russian Sturgeon, *Acipenser gueldenstaedtii* Brandt

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Karyotype structure of hybrids of crossing between sturgeon species with equal chromosome numbers, i. e. species from the same chromosome groups was examined earlier (Arefjev 1988, 1989, Burtzev *et al.* 1988, Arefjev and Philippova, unpubl. data). From mean values of quantitative karyotypic parameters hybrids either did not differ from those of the parents or were practically intermediate; disbalancing of hybrid karyotype occurred in various degrees and was accompanied by decrease of fertility and expressed as an increase of variability of the diploid number and other quantitative karyotypic parameters.

Data on hybridization of fishes under cytogenetic control are meanwhile scarce; so intermediate inheritance of karyotype characters (in particular, the diploid number) appears to be common. The more interesting should be the analysis of situation observed as a result of chromosome set study in the reciprocal hybrids between the Russian sturgeon, *Acipenser gueldenstaedtii*, and the great sturgeon, *Huso huso*.

During last 2–3 years cultivation of this hybrid has been intensively mastered in aquaculture of the U. S. S. R. It may be used in ranching programs as well as in cage-rearing both in marine and fresh water due to its easy adaptability to salinity of 16–18‰ (Burtzev *et al.* 1989). Such adaptation does not jeopardize genetic pollution of natural populations due to *a priori* supposed sterility of hybrids between the great and the Russian sturgeons.

Materials and methods

The breeders of great sturgeon and Russian sturgeon were caught in the Lower Volga River. The spawning was made artificially and the progeny was reared at the Ikryanynsk fish farm near Astrachan' in 1987. Juveniles (reciprocal hybrids and great sturgeon) at the age of 1.5–2 months were delivered to the Scientific Experimental Mariculture Station at Bolshoj Utrish (Krasnodar Region, at the Caucasus shore of the Black Sea). Viability of all variants including "pure" species and hybrids was approximately equal.

Four specimens of great sturgeon and twelve of Russian sturgeon and eleven of hybrid aged 3 months (Table 1) were used. Fishes were processed according to the Vasiliev and Sokolov method (1980) with slight modifications (Rivlin *et al.* 1985): fishes were intramuscularly injected with 0.1% colchicine ("Serva") solution in the dosage of 0.05–0.1 ml per fish (average fish weight was about 6 g). Fish was kept alive for 6–8 hr in 20–1 aquaria with intensive aeration. Tissue of head lymphoid gland was placed into hypotonic solution (0.4% KCl) for 35 min and then into cold methanol-acetic acid (3:1) fixative for 1 min and finally into fresh portion of fixative for 1 hr. Preparations were made by air-drying method on clean lipidless slides and stained with 5% Giemsa solution prepared on standard phosphate buffer (pH=6.8) for 35 min.

Metaphase plates were analyzed by light microscopy; their number is shown in Table 1 and magnification scales are presented on microphotos.

Table 1. Quantitative characteristics of karyotypes of the Russian and the great sturgeons and their reciprocal hybrids

Character	Form			
	Russian sturgeon	Russian × great	Great × Russian	Great sturgeon
No. of fishes	12	7	4	4
No. of metaphase plates	69	84	41	36
2n: mode	246–250	n/e*	n/e*	116–117
$\bar{x}$	249.9±2.2	167.2±1.6	169.4±2.9	117.6±0.4
C. V.	3.9%	8.9%	11.0%	2.7%
MC: mode	95–96	74–75	n/e*	62
$\bar{x}$	97.6±0.4	74.0±0.4	74.2±0.7	61.2±0.2
C. V.	1.9%	4.8%	5.9%	3.0%
AC: mode	151–160	91–95	n/e*	58–59
$\bar{x}$	152.2±2.6	93.0±1.4	95.3±2.4	57.6±0.6
C. V.	7.7%	14.0%	16.2%	6.0%
NF: mode	341–350	231–240	251–260	174–175
$\bar{x}$	346.1±2.3	241.3±1.9	243.6±3.5	178.2±0.5
C. V.	3.1%	7.3%	9.2%	2.2%

* n/e–mode not expressed.

Fig. 1. Metaphase plate (A) and karyogram (B) of great sturgeon: 2n=118, MC=60.

Results and discussion

Karyotype of great sturgeon (Fig. 1) appear to be identical to that of specimens from Italy (Fontana and Colombo 1974) and from the lower Volga River (Vasiliev 1985, Birstein and Vasiliev 1987). Chromosome complement of Russian sturgeon was described earlier (Arefjev 1989b), and karyotypes of the Caspian and the Black Sea populations were found to be com-

pletely similar. Chromosome sets and karyograms are presented in Figs. 2 and 3.

Hypothetically modal chromosome number in the hybrids would be intermediate, $2n = (118 + 250)/2 = 184$. However, as one can see from Table 1 and Fig. 4 the average number of

Fig. 2. Metaphase plate (A) and karyogram (B) of hybrid between great sturgeon (female) and Russian sturgeon (male): $2n = 174$, $MC = 75$.

Fig. 3. Metaphase plate (A) and karyogram (B) of hybrid between Russian sturgeon (Female) and great sturgeon (male): $2n = 153$, $NC = 69$.

chromosome appeared to be by 20 chromosomes lower than it was expected, and modes were not expressed in both hybrid variants. The same referred to MC (number of bi-armed chro-

Fig. 4. Histograms of metaphase plate number distributions for four karyotypic parameters (diploid chromosome number, 2n; number of bi-armed chromosomes, MC; number of uni-armed and microchromosomes, AC; number of chromosome arms, NF) of *Huso huso*, *Acipenser gueldenstaedtii* and their reciprocal hybrids.

mosomes): it constituted about 74–75 against 80 that was hypothetically expected. It is obvious that other quantitative parameters (numbers of uni-armed and microchromosomes, AC; and number of chromosome arms, NF) are non-intermediate in the hybrids, too.

At the same time no authentic differences between the reciprocal hybrid forms were observed in all four karyotype parameters examined (Table 1). Moreover, homogeneity of

Table 2. Karyotype structure of hybrids from crosses between fishes with different chromosome numbers

Order	Parental species and their karyotypes	Hybrid karyotype	Additional information	Source of reference
Salmoniformes	<i>Oncorhynchus keta</i> (2n=74, NF=100) × <i>O. gorbuscha</i> (2n=52, NF=100)	2n=59–68, mode with 2n=64, NF=100	—	Mitrophanov <i>et al.</i> , 1977
	<i>O. masou</i> (2n=66, NF=100) × <i>O. gorbuscha</i>	Mode with 2n=59	Sharp 2n variability (2n=50–102), often without mode	Arai, 1984
	<i>O. gasou</i> × <i>Salvelinus leucomaenis</i> (2n=84, NF=100) in reciprocal variants	Mode with 2n=75		
	<i>O. keta</i> × <i>O. masou</i> in reciprocal variants	Mode with 2n=70	Hybrids inviable	
	<i>O. keta</i> × <i>Salvelinus fontinalis</i> (2n=84, NF=100) in reciprocal variants	Mode with 2n=79	High level of aneuploidy; hybrid viability very low	Arai, 1986
	<i>Salmo salar</i> (2n=58, NF=74), <i>S. trutta</i> (2n=80, NF=100), <i>S. fontinalis</i> in all variants including reciprocal	2n near intermediate	Very high variability of 2n and bi-armed chromosome number	Gjedrem <i>et al.</i> , 1977
	<i>Salmo gairdneri</i> (2n=58–64, NF=104) × <i>S. fontinalis</i>	2n intermediate	Aneuploidy absent	Ueda <i>et al.</i> , 1984
	<i>Coregonus peled</i> (2n=72, NF=96) × <i>C. nasus</i> (2n=58, NF=92)	2n=66, 68, NF=94	—	Polyakova <i>et al.</i> , 1983
	<i>Ictalurus furcatus</i> (2n=58) × <i>I. catus</i> (2n=48)	2n=53	Portion of modal cells 50–70%	LeGrande <i>et al.</i> , 1984
	<i>I. punctatus</i> (2n=58) × <i>I. catus</i>	2n=53	Portion of modal cells ap. 40%	
Cypriniformes	<i>Cyprinus carpio</i> (2n=100) × <i>Puntius conchonius</i> (2n=50)	2n=74–76	Viability of hybrids lower than 50%	Vasiliev <i>et al.</i> , 1978
	<i>C. carpio</i> × <i>Hypophthalmichthys molitrix</i> (2n=48)	2n=74–75	Hybrid viability ap. 80%	
	<i>C. carpio</i> × <i>Tinca tinca</i> (2n=48)	2n=68–76	Hybrid viability very low	Sola <i>et al.</i> , 1983

morphological structure of karyotypes does not allow the marker chromosome to be distinguished for identification of parental karyotypes in the hybrid genome. The only thing that attracts attention is that hybrid karyotype includes only 4 relatively large acrocentric chromosomes while karyotypes of Russian and great sturgeon feature 12 and 2 such elements, respectively, which can be another result of non-intermediate karyotype inheritance.

The sharp increase of coefficient of variation (C. V.) were observed in all quantitative karyotypic parameters in both hybrids (shown in Table 1): $P < 0.001$ for $2n$, AC, NF and $P < 0.01$ for MC. Such a high variability suggests high level of karyotype disbalancing, more considerable than that described in hybrids between the equal-chromosome species: in bester (Arefjev 1989), and in hybrid between the Russian and the Siberian sturgeons (Arefjev and Phillipova, unpubl. data). Positive correlation between karyotype destabilization and fertility decrease revealed from cytogenetic analysis of various acipenserid hybrids indicates complete sterility of both reciprocal hybrids between Russian and great sturgeon as the genetically unstable forms.

Mechanisms of chromosome set variability in the hybrid examined are evidently typical for acipenserids and their hybrids and are connected mainly with aneuploidy realized with

Table 3. Dimensions of erythrocytes of the Russian sturgeon, the great sturgeon, and their hybrids

Species, form	Major axis (μm)		Minor axis (μm)		"Volume" (μm^3)	
	$\bar{x}$	C. V.	$\bar{x}$	C. V.	$\bar{x}$	C. V.
Great sturgeon*	14.22 ± 0.11	5.7%	9.63 ± 0.07	5.4%	5523.8 ± 72.6	9.3%
	13.96 ± 0.15	7.7%	9.78 ± 0.10	7.4%	5593.1 ± 96.5	12.2%
Great $\times$ Russian	14.01 ± 0.14	7.3%	11.12 ± 0.11	6.9%	7256.7 ± 125.2	12.2%
	14.36 ± 0.14	6.4%	10.80 ± 0.12	7.3%	706.0 ± 105.2	10.6%
Russian $\times$ great	14.28 ± 0.18	9.8%	10.84 ± 0.14	9.8%	7028.7 ± 153.1	15.4%
	14.57 ± 0.16	7.6%	10.63 ± 0.09	5.8%	6896.3 ± 105.3	10.8%
Russian sturgeon	17.58 ± 0.19	7.4%	13.33 ± 0.11	5.5%	13084.8 ± 181.4	9.8%
	17.39 ± 0.23	9.4%	13.34 ± 0.13	6.7%	12962.8 ± 254.8	13.9%

* for each form the data from two specimens are given

Table 4. Dimensions of erythrocyte nuclei of the Russian sturgeon, the great sturgeon, and their hybrids

Species, form	Major axis (μm)		Minor axis (μm)		"Volume" (μm^3)	
	$\bar{x}$	C. V.	$\bar{x}$	C. V.	$\bar{x}$	C. V.
Great sturgeon	6.21 ± 0.07	8.2%	4.33 ± 0.06	10.1%	487.7 ± 9.6	13.9%
	6.18 ± 0.10	11.2%	4.26 ± 0.06	9.4%	469.8 ± 11.2	16.8%
Great $\times$ Russian	6.68 ± 0.09	10.2%	5.17 ± 0.06	8.4%	747.9 ± 13.6	12.9%
	6.54 ± 0.07	7.7%	5.02 ± 0.07	9.3%	690.4 ± 13.8	14.1%
Russian $\times$ great	6.45 ± 0.11	12.2%	4.96 ± 0.08	11.9%	664.7 ± 19.3	20.5%
	6.72 ± 0.08	8.0%	4.87 ± 0.06	9.1%	667.6 ± 11.8	12.5%
Russian sturgeon	8.46 ± 0.12	9.7%	6.30 ± 0.08	9.5%	1406.5 ± 33.8	17.0%
	8.27 ± 0.09	8.0%	6.21 ± 0.07	8.4%	1325.9 ± 25.5	13.6%

microchromosomes (Arefjev 1989a). Besides, this hybrid demonstrates considerable increase in variability of bi-armed chromosome number MC: such an increase is not recorded in all three bester generations. This fact reflecting aneuploidy on bi-armed chromosomes is an additional proof for considerable karyotypic disbalancing of hybrid genome.

Data of karyotypic analysis of interspecific fish hybrids obtained from crossing between species with different $2n$ numbers are presented in Table 2; such data are available mainly on salmonids. In the majority of cases aneuploidy occurs, the degree of which may correlate with fertility rate observed in hybrids (Arai 1984). However, modes of $2n$, though not always distinctly pronounced, are often intermediate in relation to $2n$ parental karyotypes, with the exception of chromosome complements of hybrid between two cicoes, family *Coregonidae*

(Polyakova *et al.* 1983), where $2n$ is higher than intermediate value, and hypomodal karyotype of hybrid between carp and tench (Sola *et al.* 1983).

Non-intermediate inheritance of chromosome number is occasionally noted in other groups of animal, for example, in hybrids between *Drosophila nasuta nasuta* ($2n=6$) and *D. n. albomicana* ($2n=8$) which shows $2n=6$ and $2n=7$ (Ramachandra and Ranganath 1986). On the whole, rarity of non-intermediate karyotype patterns indicates high degree of karyotypic destabilization of such hybrids and consequently their complete sterility and, in some cases, low viability.

Genome size (moreover, chromosome number) can be estimated indirectly, in particular, by cell size measurements, and the analysis of erythrocyte dimensions appears to be the most expedient. Data of such measurements are shown in Tables 3 and 4. "Volumes" of erythrocytes and their nuclei are calculated from the equation $V=4/3\pi \cdot a \cdot b^2$, where a and b are major and minor axis length.

None of the forms is distinguished from other in terms of C. V. of all characters: they are likely to be too "rough" to reflect increasing aneuploidy of hybrid karyotype. At the same time average values of erythrocyte and nucleus linear sizes of great sturgeon and hybrids do not considerably differ, while erythrocytes of Russian sturgeon are prominently larger (Fig. 5). Such a difference is evidently conditioned by di-tetraploid relationship between these species. In particular, this correlation is used for indirect testing of ploidy level in salmonids based on erythrocyte dimension measurement (Benfey and Sutterlin 1984). This method may be recommended for similar experiments on acipenserids in the U. S. S. R..

By erythrocyte and nucleus volume the hybrids are evidence for a displacement of patterns towards those of the great sturgeon, which can serve as confirmation of non-intermediate inheritance of genome structure in the hybrid examined.

Thus, the data presented demonstrate strong destabilization of genetic apparatus in hybrid between the great and the Russian sturgeon quantitatively being the highest among karyotyped acipenserids and their hybrids. It is precisely this fact that allows for prediction of complete sterility of this hybrid.

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Summary

Hybrids between the great sturgeon and the Russian sturgeon in reciprocal crosses are found to exhibit non-intermediate chromosome number, $2n=167-169$, on average. The hybrids are also characterized by sharp increase in variability of the quantitative karyotypic parameters such as diploid chromosome number, bi-armed chromosome number, number of

Fig. 5. Circulating erythrocytes from blood of *Acipenser gueldenstaedtii* (a), *Huso huso* (b), and their hybrid (c).

uni-armed and microchromosomes, and chromosome arm number. Measurements of erythrocytes and their nuclei confirmed the di-tetraploid relationship of the parental species and non-intermediate pattern of the hybrid genome. Sharp disbalancing of karyotype of both reciprocal hybrids is indicative of high probability of their complete sterility.

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