

in  $^3\text{H}$ -PL binding between the IN-treated and the control karyotypes. The probable error computed for several groups of karyotypes corresponding to different conditions of treatment was between 15 to 30% of the mean  $G_c-G_c$  values.

The  $G_c-G_c$  values for A group chromosomes are given in the Table. The most striking effect of IN-treatment was on  $A_2$  chromosome. It did bind less  $^3\text{H}$ -PL than the control  $A_2$  chromosome under nearly all conditions of IN-treatment. It was also observed that there was a dependence of  $^3\text{H}$ -PL binding on the degree of chromosomal contraction ( $\Sigma \Delta G$  values). A long  $A_2$  chromosome exposed to IN for 4 h did bind more  $^3\text{H}$ -PL than a short  $A_2$  chromosome. The opposite result was obtained for the 48 h exposure time. In addition, there was observed for  $A_2$  chromosome a gradual decrease of  $\Sigma \Delta G$  with the exposure time for each IN dose employed.

The results obtained for A group chromosomes, particularly for  $A_2$  chromosome, suggest that the organization of chromosomal nucleic acids and proteins might be different in IN-treated chromosomes than in controls. The  $^3\text{H}$ -PL binding would be expected to occur most readily in those regions of metaphase chromosomes that contain exposed segments of nucleic acids. The binding occurs through ionic interactions between positively

charged  $\epsilon$ -amino groups of  $^3\text{H}$ -PL and negatively charged phosphodiester linkages of nucleic acids. At least 10 to 20 such interactions would be required per one  $^3\text{H}$ -PL molecule to maintain a stable complex, since the interaction energy for one mole of ion pairs is of the order of 1 Kcal.

**Zusammenfassung.** Nachweis, dass die Bindung von tritiummarkiertem Poly-L-Lysin ( $^3\text{H}$ -PL), mit den Chromosomen der A-Gruppe (speziell mit dem  $A_2$ -Chromosom) aus isoniazidhaltigen Kulturen der menschlichen Lymphocyten isoliert, verschieden von der Bindung desselben mit nicht behandelten Chromosomen war. Das Bindungsvermögen des behandelten  $A_2$ -Chromosoms ist überdies von der Chromosomenlänge abhängig.

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## The Chromosomes of Italian Sturgeons

The cytogenetics of the Acipenseridae is interesting for two main reasons: 1. they are rather primitive fishes; 2. their taxonomy is complicated; many interspecific and intergeneric hybrids are described<sup>1</sup> and artificially obtained<sup>2</sup>.

The only careful description of the karyotype so far published, to our knowledge, is that by OHNO et al.<sup>3</sup> of the shovelnose sturgeon, *Scaphirhynchus platyrhynchus*, which has about 112 chromosomes (diploid number) with about 48 dot-like microchromosomes. The karyotypes of *Huso huso* L., *Acipenser ruthenus* L., and their hybrid were studied by SEREBRYAKOVA<sup>4</sup>; but the microchromosomes were not noticed and complements of only 60 chromosomes were described for both species and their

hybrids. Also for the *A. stellatus* Pall. and *A. nudiiventris* Lov. karyotypes of 60 chromosomes were described, whilst *A. güldenstädti* Br. should have more than 130 chromosomes<sup>2</sup>.

In Northern Italy, 3 species are present<sup>5</sup>: *Huso huso* L., *Acipenser sturio* L. and *Acipenser Naccarii* Bonaparte; this last species is found only in the North Adriatic. We studied the karyotypes of some specimens of these 3 species, collected from the Po river during spring and autumn 1973.

<sup>1</sup> L. S. BERG, *Freshwater Fishes of the U.S.S.R. and Adjacent Countries* (Israel Program for Scientific Translations, Jerusalem, translated from Russian 1962).

<sup>2</sup> N. I. NIKOLYUKIN, *Genetika* 5, 25 (1966).

<sup>3</sup> S. OHNO, J. MURAMOTO, C. STENIUS, L. CHRISTIAN, W. A. KITRELL and N. B. ATKIN, *Chromosoma* 26, 35 (1969).

<sup>4</sup> E. V. SEREBRYAKOVA, in *Genetics, Selection, and Hybridization of Fish* (Ed. B. I. CHERFAS; Israel Program for Scientific Translations, Jerusalem, translated from Russian, 1962), p. 98.

<sup>5</sup> U. D'ANCONA, Ministero Econ. Naz., Ufficio Pesca (1924).

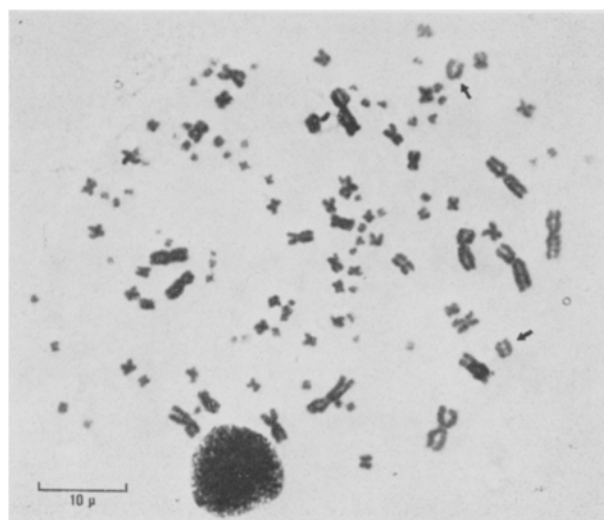


Fig. 1. Metaphase plate of *Huso huso*, arrows indicate the pair of large acrocentric chromosomes.

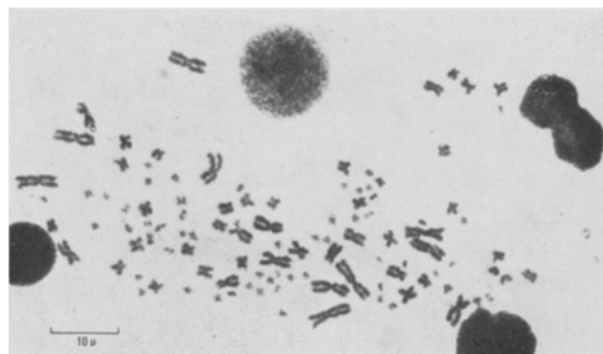


Fig. 2. Metaphase plate of *Acipenser sturio*.

Distribution of diploid chromosome counts obtained from 3 species of Acipenseridae

Species	Sex	No.	Diploid chromosomes					
			101-110	111-120	121-130...	221-230	231-240	241-250
<i>Huso huso</i>	♂	1	—	10	2			
	♀	2	2	26	2			
	Not det.	2	4	14	1			
<i>Acipenser sturio</i>	♀	2	5	37	13			
<i>Acipenser Naccarii</i>	♂	1				3	5	11
	♀	3				6	11	20

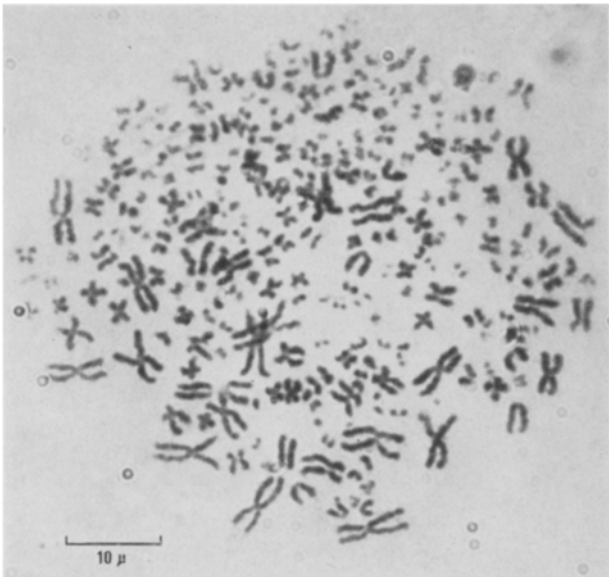


Fig. 3. Metaphase plate of *Acipenser Naccarii*.

The technique used was a modified version of that reported by CAPANNA et al.<sup>6</sup>. Each fish was injected either i.m. or s.c. with 0.3 ml/100 g of Colcemid Ciba. 3 h later, the injected fish was killed; the 'head' kidney was removed, cut to pieces of approximately 0.5 mm<sup>3</sup> and placed in a hypotonic sodium citrate solution for 20 min; this solution was removed and a cold fixative (3:1 mixture of ethanol and glacial acetic acid) was added. The material was centrifuged and after 3 successive fixations, the final cell suspension was squashed on chilled slides according to the air-drying method and stained by the Giemsa solution. Sex of each fish was determined by histological examination of the gonads. 9 to 17 metaphases per individual were examined of 5 specimens of *H. huso*, 4 to 32 of 4 *A. Naccarii*, 27 and 28 metaphases of 2 *A. sturio*.

The results are reported in the Table. The diploid chromosomes numbers are: *H. huso* 116 ± 4, *A. sturio* 116 ± 4, *A. Naccarii* 239 ± 7. The karyotypes of the 3 species are reported in the Figures 1-6. All the karyotypes are characterized by a large group of meta- and

<sup>6</sup> E. CAPANNA, S. CATAUDELLA and R. VOLPE, Boll. Pesca Piscic. Idrobiol. 26, 245 (1971).

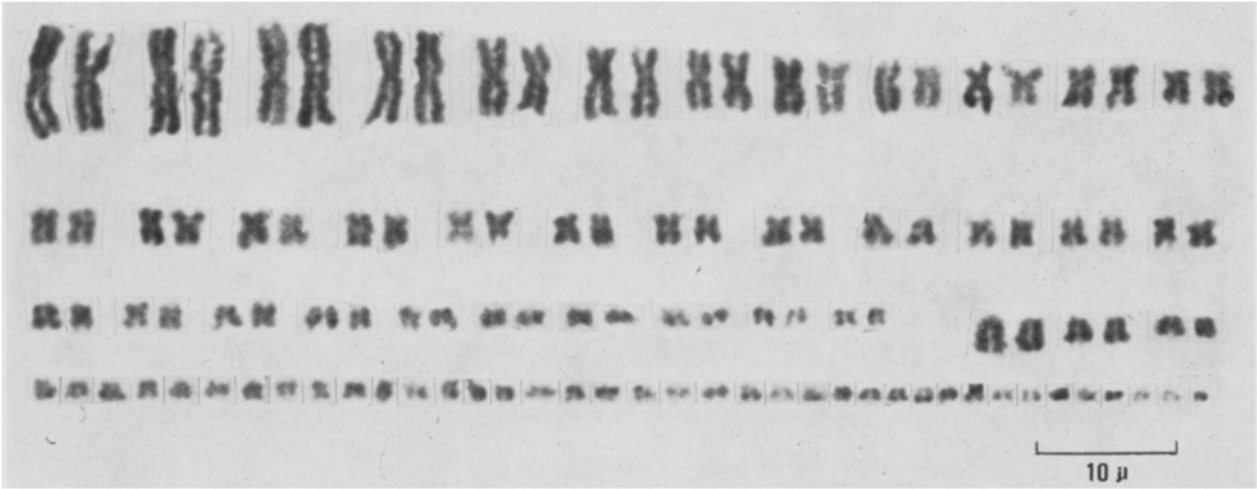


Fig. 4. Karyotype of *Huso huso*. The meta- and submetacentric chromosomes were first aligned in order of declining size, followed by the acrocentric and finally the microchromosomes.

submetacentric chromosomes, few acrocentrics, and a large number of dot-like microchromosomes. It is difficult to distinguish among the small chromosomes the meta- and submetacentric ones, and to separate the small chromosomes from the dot-like microchromosomes, because the size of the chromosomes decreases in a rather

continuous pattern. However, it is possible to characterize each karyotype by taking into account mainly the larger chromosomes. The diploid complement of *H. huso* consists of 34 pairs of meta- and submetacentric chromosomes, 3 pairs of acrocentrics, 1 long and 2 shorter, and about 38 microchromosomes. *A. sturio* has 35 pairs of meta- and sub-

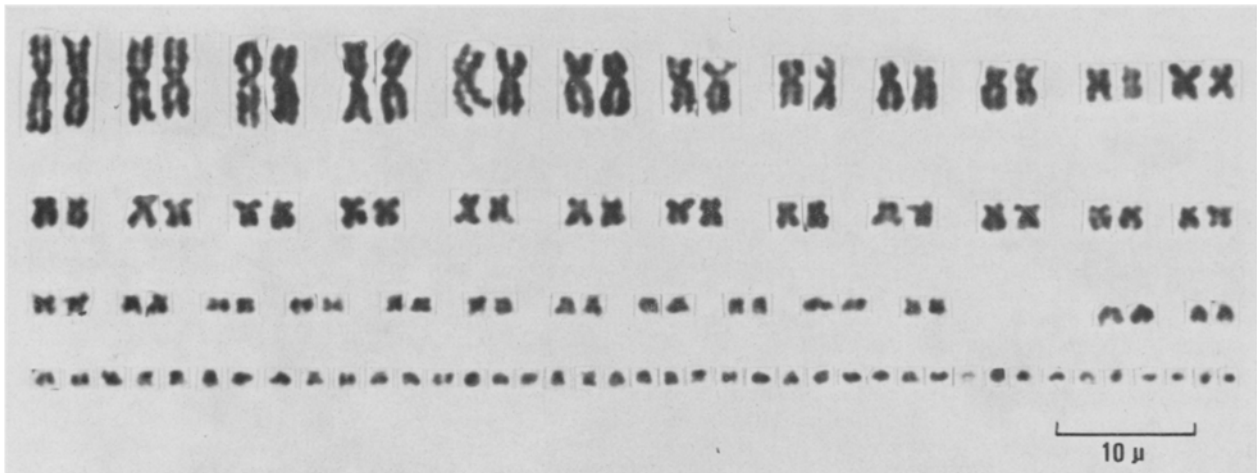


Fig. 5. Karyotype of *Acipenser sturio*. The meta- and submetacentric chromosomes were first aligned in order of declining size, followed by the acrocentric and finally the microchromosomes.

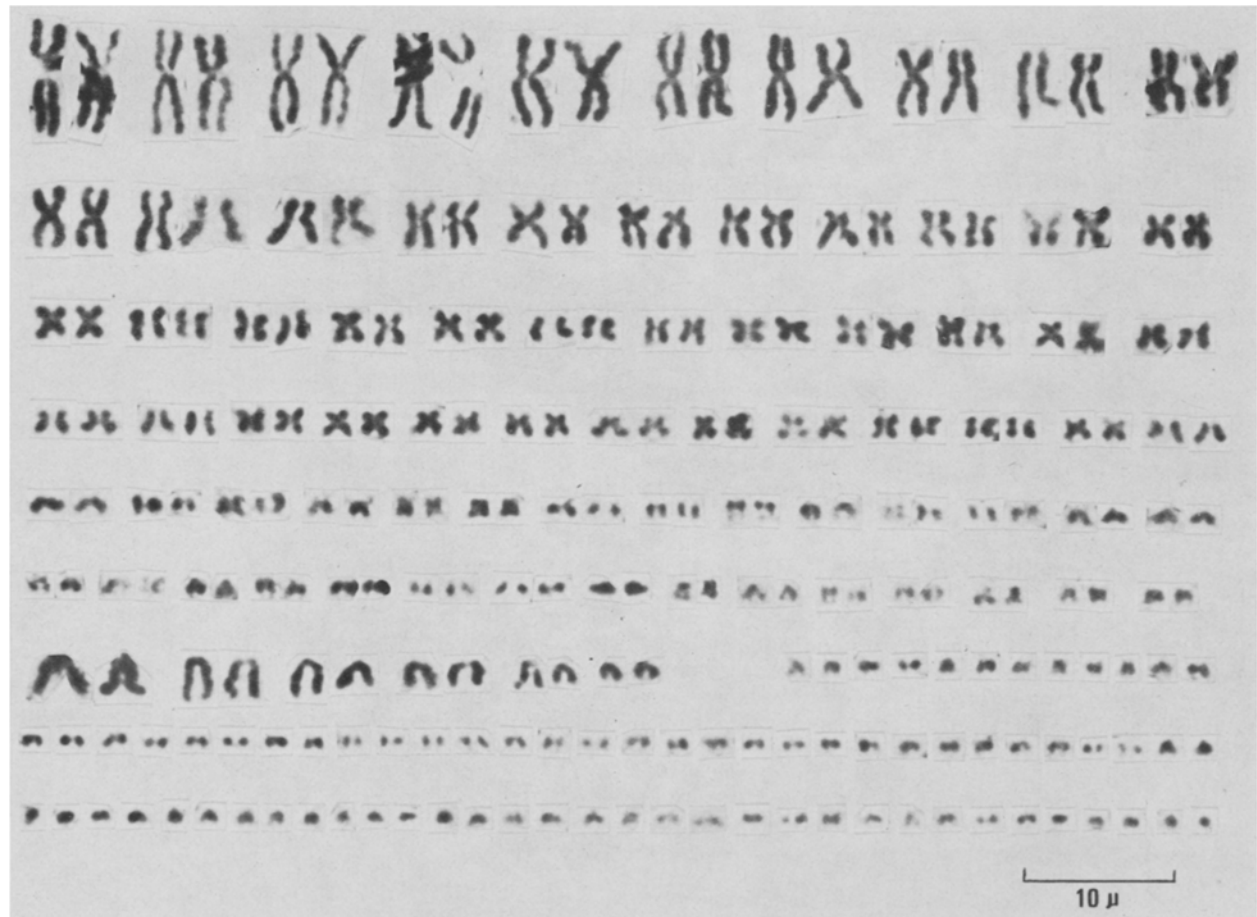


Fig. 6. Karyotype of *Acipenser Naccarii*. The meta- and submetacentric chromosomes were first aligned in order of declining size, followed by the acrocentric and finally the microchromosomes.

metacentric chromosomes, only 2 pairs of small acrocentrics and about 40 microchromosomes. The karyotype of *A. Naccarii* consists of 75 pairs meta- and submetacentric, 6 pairs of acrocentric and about 76 microchromosomes. Although the number of chromosomes is the same, the karyotypes of *H. huso* and *A. sturio* are different and easily distinguishable by the presence in *H. huso* of a pair of large acrocentric which is lacking in *A. sturio*. *A. Naccarii* appears to be a polyploid, and by considering the morphology of the larger chromosomes, its karyotype differs from those of both *H. huso* and *A. sturio*.

The karyotypes of the above species of Acipenseridae are characterized by a high chromosomes number and by a large number of microchromosomes, similar to that found by OHNO et al.<sup>3</sup> for *S. platyrhynchus*; this condition seems to be valid for the whole family of these Chondrostei. OHNO<sup>3</sup> also puts the question whether the high chromosome number of sturgeons is due to poliploidy; our results show that the number  $2n =$  about 116 is widely distributed and perhaps it is not a polyploid condition; probably polyploid species, such as *A. Naccarii*, with about 240 chromosomes do exist.

The Russian authors<sup>2,4</sup> seem not to have noticed the microchromosomes; by considering only the larger meta- and submetacentric chromosomes, the karyotypes they describe are similar to those reported here. Therefore we believe that also *A. stellatus*, *A. nudiventris* and *A. ruthenus* have a fundamental chromosome number of about 120 similar to *H. huso*, *A. sturio* and *S. platyrhynchus*; while *A. güldenstädti* has a complement of about 240 chromosomes like *A. Naccarii*, and both might be polyploids.

Many species of Acipenseridae produce easily interspecific and intergeneric hybrids<sup>1,2</sup>; the taxonomy of these species should be revised. The cytogenetic approach could provide a contribution to these problems. A working hypothesis to be tested is that *H. huso*, *A. stellatus*, *A. ruthenus* and *A. nudiventris*, which produce

fertile hybrids and also have similar karyotypes characterized by the presence of 3 acrocentric chromosomes, may be strictly related species or even the same species. Since *A. güldenstädti* and *A. Naccarii* might both be polyploid, the comparison of their karyotypes could throw light on their reciprocal taxonomical relationship. *H. huso* and *A. güldenstädti* produce unfertile hybrids<sup>2</sup>. There is at present no available evidence on the derivation of *A. güldenstädti* and *A. Naccarii* as polyploid species; the morphology of the larger chromosomes of *A. Naccarii* differs from that of *H. huso* and *A. sturio*.

**Résumé.** On présente l'étude caryologique des trois espèces d'Esturgeons des eaux italiennes. L'espèce *Huso huso* a un nombre diploïde d'environ 116, dont 34 couples de chromosomes métacentriques et submetacentriques, 3 couples de chromosomes acrocentriques, et environ 38 microchromosomes. L'espèce *Acipenser sturio* est caractérisée par environ 116 chromosomes, dont 35 couples métacentriques et submetacentriques, 2 couples de petits acrocentriques, et 40 microchromosomes. L'espèce *Acipenser Naccarii* est caractérisée par 240 chromosomes environ, soit, 75 couples de chromosomes métacentriques et submetacentriques, 6 couples d'acrocentriques et environ 78 microchromosomes. Les données caryologiques sont confrontées avec la systématique des Acipenseridae.

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## Oceanian Type Black Rats (*Rattus rattus*) with a Subtelocentric $M_2$ Chromosome and C-Type Transferrin Obtained from North America

Oceanian type black rats with 38 chromosomes have been found widely in the world, namely in Oceania, South America, Europe, North America, Central Asia, Southwest Asia and Africa. The idiogram of these rats differs markedly from that of the Asian type with 42 chromosomes, by having 2 large metacentric pairs. According to YOSIDA et al.<sup>1-3</sup>, the larger metacentric pair ( $M_1$ ) have originated in the Robertsonian fusion of acrocentric pairs No. 4 and 7 and the other smaller one ( $M_2$ ) in that of the acrocentric pairs No. 11 and 12 in the Asian type black rat. Sizes of 2 arms of the  $M_2$  chromosome in these rats are usually similar. However, a subtelocentric  $M_2$  chromosome, which is remarkable by having a shorter arm, was found in the black rats obtained from North America. In this paper the idiogram of these rats will be reported with special interest in the origin of the subtelocentric  $M_2$  chromosome. In addition, electrophoretic analysis of their sera was carried out, indicating Oceanian type transferrin in those rats and also suggesting the homogeneous nature of the breeding colony.

The black rats (*Rattus rattus*) used in the present study were kindly supplied from Dr. REX MARSH and Mr. RAY RECORD in the Department of Animal Physiology, University of California at Davis, when we visited

there in August, 1973. According to Dr. MARSH's correspondence 3 pairs of black rats were collected in San Lorenzo, California in 1970, by Mr. VAL DUTSON of Public Health in Berkeley, and since then they have been bred in an outdoor population cage. 10 rats (5 females and 5 males), randomly caught from the breeding colony, were given to us to send to Japan.

Chromosomes of these rats were observed in short-term cultured cells from their tail tips following the procedure described in the previous papers<sup>4</sup>. Chromosome preparations were made by conventional air drying technique and stained with Giemsa solution. To observe the banding patterns of chromosomes, SDS technique<sup>5</sup> was applied. In cutting the tail tip, a small blood sample, approximately 0.5 ml, was obtained for electrophoretic

<sup>1</sup> T. H. YOSIDA, K. TSUCHIYA, H. T. IMAI and K. MORIWAKI, Jap. J. Genet. 44, 89 (1969).

<sup>2</sup> T. H. YOSIDA, K. TSUCHIYA and K. MORIWAKI, Chromosoma 33, 252 (1971).

<sup>3</sup> T. H. YOSIDA and T. SAGAI, Chromosoma 37, 387 (1972).

<sup>4</sup> T. H. YOSIDA, K. TSUCHIYA and K. MORIWAKI, Chromosoma 33, 30 (1971).