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Practical Use of Cytogenetics in Fish Biology and Aquaculture

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Key words: fish cytogenetics, taxonomy, phylogeny, chromosomal polymorphism, chromosome set manipulation, hybridization

Abstract

The reviewed information was accumulated from our current and earlier cytogenetic studies on various commercially important fish species. The major emphasis of the review is to show in which way classical cytogenetics can be practically used for basic and applied research in fish biology and aquaculture.

Asian Pacific Salmon

Taxonomy, evolutionary relationships, and chromosomal polymorphism were studied in economically important Asian representatives of the Pacific salmon (genus *Oncorhynchus*). A wide karyotypic diversification ($2n = 52$ to 74) was found to be a particular characteristic of this tetraploid group of fish (Gorshkova, 1978). Among species of the genus *Oncorhynchus*, analyses of phylogenetic relationships were based on thorough studies of their karyological patterns. As a result, we could validate the existence of two subgroups within the genus. One consists of chinook (*O. tshawytscha*), coho (*O. kisutch*), and masu (*O. masu*) salmon with karyotypes of at least 14 subtelocentric chromosomes. The other

consists of pink (*O. gorbuscha*), sockeye (*O. nerka*), and chum (*O. keta*) salmon with 2-6 subtelocentric chromosomes in their karyotypes (Gorshkova, 1978, 1980). This finding was consistent with the combined data set on their biology, ecology, and external and internal morphology (Gorshkov and Gorshkova, 1981a).

Later on, our assumption received strong support from studies of mitochondrial DNA sequences and one nuclear growth hormone introne (McKay et al., 1996). In addition, we conducted analyses and thorough examinations of the existing karyotypes in numerous Kamchatkian (Gorshkova and Gorshkov, 1985) and North American representatives of

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Pacific trout, subgenus *Parasalmo* according to the nomenclature as suggested by Vladykov and Gruchy (1972). Results showed that there are many subspecies within Pacific trout that differ markedly in diploid number, number of subtelocentric chromosomes, and chromosome arms (NF). Based on these karyotype features, we proposed that the main members of Pacific trout can be divided into two phylogenetically different subgroups. One subgroup includes *Salmo gairdneri*, *S. aguabonita*, *S. mykiss*, *S. clarki henshawi*, and *S. c. bouvieri*, characterized by only one pair of subtelocentric chromosomes. The other subgroup includes subspecies of *S. clarki clarki* and *S. c. lewisi* with 12-18 subtelocentric chromosomes. The classification of morphological chromosome structure was carried out according to the position of the centromere as outlined by Levan et al. (1964).

Another project focused on studying the intraspecific structure in members of the Pacific salmon. For example, cytological examination of sockeye salmon revealed considerable differences between seasonal races of *O. nerka* (spring and fall races) inhabiting Lake Azabachye (Kamchatka). The modal karyotype of the spring form contained $2n = 58$ chromosomes and $NF = 104$, while the fall sockeye salmon contained $2n = 56$ and $NF = 102$. In addition, intra-individual variability in diploid number was detected in both races. We suggested that intraspecific differentiation in *O. nerka* could arise in different spawning grounds in the lake and occurred independently due to the absence of any migrations between brood fish of seasonal races during many generations (Gorshkova and Gorshkov, 1978; Gorshkov et al., 1985).

Intraspecific chromosomal polymorphism was found to be a widespread genetic phenomenon in Kamchatkian and Sakhalin populations of pink salmon (*O. gorbuscha*). Among fish of this species we found three major karyotypes, consisting of 52, 53, or 54 chromosomes with a constant NF of 104. The karyotype with $2n = 54$ and four acrocentric chromosomes was supposed to be phylogenetically an initial one while the karyotype of $2n = 53$ was heterozygous. The $2n = 52$ karyotype

was homozygous, resulting from two centric fusions of acrocentric chromosomes of the Robertsonian type. In meiosis of adult fish, heterozygous males had more varied balanced and unbalanced gametes than homozygous males. Significant differences in the frequency of karyological morphs ($2n = 52, 53, 54$) were found between odd and even-year populations of pink salmon (Gorshkov and Gorshkova, 1981b; Gorshkova and Gorshkov, 1983). Heterozygotes dominated in odd-year populations, whereas homozygous fish with $2n = 52$ prevailed in even-year populations. A close correlation was found between mortality rates in early embryonic stages and levels of chromosomal aberrations. In general, our results showed the existence of pre-zygotic mechanisms of natural selection that could affect survival rates and dynamics, abundance of sympatric even and odd generations that are completely isolated groups due to the two-year life cycle of this species, and total death after spawning. Accordingly, pink salmon would be considered as comprising the most divergent temporal races among salmonids (Gorshkov et al., 1985; Gorshkova et al., 1986, 1988). These conclusions were used to develop practical tools for sustainable fisheries of Asian stocks of Pacific salmon.

In Pacific salmon, there were attempts to investigate the performance of interspecific hybrids. We found that some individuals among the hybrids had morphologically normal and even mature male gonads (for example, pink x chinook reciprocal hybrids), whereas other fish had abnormal and completely sterile gonads. Morphological, histological, and karyological analyses produced evidence that reproductive barriers between such species as chum and pink, pink and chinook salmon are incomplete. These data can be of importance for conservation biology, ecological geneticists, and state environmental and fishery management agencies (our unpublished data).

A program aiming at application of chromosome set manipulation in rainbow trout aquaculture was initiated in the mid 1980s in the Baltic Sea region of the former USSR.

Studies included comparative cytogenetic estimation of viability of various rainbow trout strains cultured in various hatcheries, optimizing triploidy induction on a commercial scale, and research on induced diploid gynogenesis in combination with hormonal masculinization. As a result, we detected the main types and frequencies of chromosomal aberrations in embryos of different rainbow trout strains. The strains with the best performance were suggested as candidates for commercial rearing and mass production of triploids and gynogenetic offspring (Gorshkova and Gorshkov, 1991; Gorshkov and Gorshkova, 1992). Some of our recent studies dealt with refining techniques for chromosome set manipulations in Pacific salmon. This data can be helpful for developing techniques for sterilization of transgenic forms in cultured fish (our unpublished data).

Mediterranean Marine Species

A large-scale cytogenetic project was conducted with commercially important marine fish species reared at the National Center for Mariculture (NCM, Eilat, Israel). In the early 1990s, the NCM was the first to organize pioneering experiments and develop practical techniques for chromosome set manipulation aimed at genetic improvement of the gilthead sea bream, *Sparus aurata*, and the European sea bass, *Dicentrarchus labrax* (Gorshkova et al., 1995). Chromosome set manipulation techniques have a variety of uses in practical fish genetics: rapid development of inbred lines and subsequent crossbreeding, production of all-female populations, production of sterile fish, and increased growth in triploids or hybrids (Gorshkova et al., 1998).

For *S. aurata*, we established the basic conditions for producing gynogenetic and triploid individuals. The three main parameters for retention of the second polar body were time after fertilization for shock treatment (3 min), shock temperature (36.5-37.5°C), and shock duration (2.5 min). Triploidization efficiency in embryos positively correlated with shock temperature, reaching a maximum of 100% at 37°C. Verification of gynogenetic and triploid production was obtained using UV-irra-

diated sperm from the Japanese red sea bream (*Pagrus major*) and through karyological examination of the embryos and larvae. Diploid numbers (2n) of parental species and their diploid hybrids possessed 48 chromosomes. The karyotype of *S. aurata* had six metacentric and not less than eight subtelocentric chromosomes, with $NF = 54+8m$ (where m is the short arm of the subtelocentric chromosomes). The karyotype of *P. major* consisted of 46 acrocentric and only 2 subtelocentric chromosomes, with $NF = 48+2m$. The karyotype of reciprocal diploid hybrids between these species had three metacentric, five subtelocentric, and 40 acrocentric chromosomes with $NF = 51+5m$. The metacentric (bi-armed) chromosomes derived from the *S. aurata* karyotype were easily detected in hybrids as "marker" chromosomes. Therefore, in the karyotype of produced triploid ($3n = 72$) hybrids there were six easily detectable "marker" metacentric chromosomes derived from two maternal (*S. aurata*) complements (Gorshkova et al., 1995; Gorshkov et al., 1998). The use of sperm from *P. major* and, subsequently, from the Red Sea double bar sea bream, *Acanthopagrus bifasciatus* (Sparidae), enabled karyological differentiation of the gynogenetic, diploid, and triploid hybrid offspring. Interestingly, hybrids resulted from artificial crosses between female *S. aurata* and male *A. bifasciatus* had high fertilization and hatching rates but no hybrid fish survived beyond day 30, suggesting considerable reproductive barriers between these species in the wild (Gorshkova et al., 1998). Thus, it was found that males of the double bar sea bream could be very useful for mass production of chromosomally manipulated forms of the gilthead sea bream. Viable hybrids between the European sea bass (*D. labrax*) females and striped bass (*Morone saxatilis*) males were also obtained using 8 ppt seawater to activate *M. saxatilis* sperm (the striped bass, *M. saxatilis*, was introduced to Israel from America in the mid 1990s). Surprisingly, karyological analysis of fish that survived six months showed that all examined individuals were triploid hybrids (Knibb et al., 1998).

Meiotic gynogenetic progeny of the

European sea bass were produced using both cold and heat shocks to eggs fertilized with UV-irradiated sperm from sea bass males (Gorshkova et al., 1995; Knibb et al., 1998). In these experiments, mass karyotyping of embryos and larvae was conducted to compare techniques of sperm inactivation and karyological specificity during transition from different ploidy levels in chromosomally manipulated offspring. In addition, to verify ploidy levels in chromosomally manipulated forms, we used cytometric analyses of erythrocytes. Thorough karyological examinations were carried out on females and males of sea bass. The karyotype studied in different strains of *D. labrax* was composed of 46 acrocentric and two small subtelocentric chromosomes. Males usually had a heteromorphic pair of these subtelocentric chromosomes, whereas the majority of the females had a homologous pair of subtelocentric chromosomes and this sex-associated difference was highly significant ($p < 0.001$; Gorshkova et al., 1996a).

Intraspecific chromosomal polymorphism was also found in hatchery strains (NCM) of the gilthead sea bream. It is likely that it will be possible to identify particular cytogenetic features responsible for the unique Mendelian mutations named “ebony” and “yellow” among sea bream strains reared at the NCM. Presently, we are collaborating with fish genetics laboratories in Europe (within the framework of an EC cooperative project) on a major initiative to map the sea bream genome. For this, using previously developed techniques, we produced gynogenetic progeny of the gilthead sea bream that were used to develop the first genome map for sea bream.

Other Species

Cytological techniques were adaptable and very useful for examination of early embryogenesis in cultured marine fish. A study aimed at understanding the genetic reasons for high egg and larval mortality in the commercially important white grouper, *Epinephelus aeneus*. The proportion of cytogenetically abnormal embryos carrying different types of chromosomal aberrations varied significantly among spawnings of the parental fish and ranged

35.5-79%. We proposed that chromosomal disorders might be one of the particular genetic factors affecting survival during the embryonic and early development stages. Analysis of chromosomal aberrations can also be a valuable tool to assess mutagenic effects of some pollutants that are chromosome-damaging agents (Gorshkova et al., 2002a).

Several cytogenetic studies were conducted on cultured and wild freshwater fish species. In the mid 1990s, we performed karyological examinations on sturgeons that were imported from Russia to Israel and reared at the Kibbutz Dan fish farm as a subject for experimental culture. Karyological analysis was conducted on hybrids between the Russian sturgeon (*Acipenser guldenstadtii*) and the great (beluga) sturgeon (*Huso huso*). Results showed that the consistent mode of $2n$ was 181-190 and the karyotype comprised 78 metacentric and submetacentric, 16 acrocentric, and about 88 microchromosomes. The data confirmed the intermediate origin of the imported hybrid that resulted from the species with a markedly different number of chromosomes, suggesting the polyploid origin of the family Acipenseridae. Thus, karyological analysis provided a reliable identification of the imported fish and allowed us to make recommendations regarding conservation of these stocks in Israel (Gorshkova et al., 1996b). In subsequent studies, we proposed that the application of combined cytometric and karyological techniques could be useful for genetic management of cultured sturgeon stocks in Israel (work in progress).

A cooperative study (with the Department of Systematics and Evolution of the Hebrew University of Jerusalem) in the area of fish systematics and phylogeny was recently conducted on two endemic Israeli freshwater species that inhabit the basin of Lake Kinneret (Sea of Galilee, Israel): the large scaled barbel *Barbus canis* and the small scaled Damascus barbel *Capoeta damascina* (Cyprinidae). The karyotypes of these species, belonging to the geographical group “Levantine barbels”, had never been described. For both species, consistent

modes of diploid numbers were 148-150 chromosomes, with NF = 224-228. Since these fish have a polyploid origin and complicated taxonomy, the results allow a better understanding of their systematics and phylogenetic relationships (Gorshkova et al., 2002b).

Conclusions

In fish biology and aquaculture, the use of classical cytological tools may be very informative and useful in studies on: (a) systematic and taxonomic relationships, (b) chromosomal polymorphism, (c) interspecific hybridization, (d) early embryogenesis, (e) chromosome set manipulations, and (f) sex determination mechanisms.

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References

- Gorshkov S. and G. Gorshkova**, 1981a. Analyses of relationships between species of Pacific salmon of the genera *Oncorhynchus* and *Salmo*. *Zoologia*, 1:84-95 (in Russian with English summary).
- Gorshkov S. and G. Gorshkova**, 1981b. Chromosomal polymorphism of the pink salmon, *Oncorhynchus gorbuscha*. *Cytology*, 8:954-960 (in Russian with English summary).
- Gorshkov S. and G. Gorshkova**, 1992. Application of chromosome manipulations in aquaculture of rainbow trout in the Baltic Sea region, USSR. *Aquaculture*, 100:99-100.
- Gorshkov S., Kurenkov S., Vronsky B. and I. Vetzler**, 1985. Sympatric intraspecific forms of Kamchatkian salmonids. *J. General Biol.*, 5:645-654 (in Russian with English summary).
- Gorshkov S., Gorshkova G., Hadani A., Gordin H. and W. Knibb**, 1998. Chromosome set manipulations and hybridization experiments in gilthead sea bream (*Sparus aurata*). I. Induced gynogenesis and intergeneric hybridization using males of the red sea bream (*Pagrus major*). *Isr. J. Aquac.* - *Bamidgeh*, 50:99-110.
- Gorshkova G.**, 1978. Some of peculiarities of karyotypes of Pacific Salmon. *Cytology*, 20:1431-1435 (in Russian with English summary).
- Gorshkova G.**, 1980. Karyology and chromosomal polymorphism of Pacific salmon. In: V.S. Kirpichnikov (ed.). *Karyological Variability, Mutagenesis and Gynogenesis in Fish*. Inst. Cytology, Acad. Sci. USSR, Leningrad, USSR. 95 pp.
- Gorshkova G. and S. Gorshkov**, 1978. Chromosome sets in seasonal races of the sockeye salmon, *Oncorhynchus nerka* (Walb.) in the Azabachye Lake (Kamchatka). *Zoologia*, 9:1342-1358 (in Russian with English summary).
- Gorshkova G. and S. Gorshkov**, 1983. Chromosomal polymorphism of the pink salmon of even and odd years. *Dokl. Acad. Sci. USSR*, 4:1023-1025 (in Russian).
- Gorshkova G. and S. Gorshkov**, 1985. Karyotypes of the Kamchatkian salmonids of the genus *Salmo*. *Vop. Ichthyologii*, 4:553-560 (in Russian). *J. Ichthyol.*, 4:115-123 (in English).
- Gorshkova G. and S. Gorshkov**, 1991. Cytogenetic estimation of survival in progeny of rainbow and Donaldson's trout. pp. 68-74. In: C.B. Rusakavichus (ed.). *Aquaculture in the Baltic Sea*, Avots Press, Riga, Latvia (in Russian).
- Gorshkova G., Gorshkov S., Chebanov N. and N. Ezkova**, 1986. Estimation of the vitality of even- and odd-year broods of pink salmon, *Oncorhynchus gorbuscha* (Walb.) (Salmonidae) using the level of cytogenetic aberrations at early stages of embryogenesis. *Genetika*, 9:2339-2346 (in Russian with English summary).
- Gorshkova G., Gorshkov S. and N. Kynas**, 1988. Chromosomal polymorphism of the pink

salmon from the populations of even-odd year brood-lines. *Genetika*, 10:1873-1881 (in Russian with English summary).

Gorshkova G., Gorshkov S., Hadani A., Gordin H. and W. Knibb, 1995. Chromosome set manipulations in marine fish. *Aquaculture*, 137:157-158.

Gorshkova G., Gorshkov S., Gordin H. and W. Knibb, 1996a. Sex control and gynogenetic production in the European sea bass, *Dicentrarchus labrax*. pp. 288-290. In: B. Chatain, B. Saroglia, M. Sweetman, P. Lavens (eds.). *Sea Bass and Sea Bream Culture: Problems and Prospects*. International Workshop, October 16-18, 1996, Verona, Italy. Eur. Aquac. Soc., Ostende, Belgium.

Gorshkova G., Gorshkov S., Gordin H. and W. Knibb, 1996b. Karyological studies in hybrids of Beluga *Huso huso* (L.) and the Russian sturgeon *Acipenser guldenstadti* Brandt. *Isr. J. Aquac. - Bamidgeh*, 48:35-39.

Gorshkova G., Gorshkov S., Gordin H. and W. Knibb, 1998. Chromosome set manipulation and interspecific hybridization in marine fish (Sparidae, Serranidae). *Isr. J. Aquac. - Bamidgeh*, 50:210-211.

Gorshkova G., Protas Y, Ben-Atia S. and S. Gorshkov, 2002a. Cytogenetic examination of early embryonic development in the white grouper, *Epinephelus aeneus* (Pisces, Serranidae). *J. Appl. Ichthyol.*, 18:29-34.

Gorshkova G., Gorshkov S. and D. Golani, 2002b. Karyotypes of *Barbus canis* and *Capoeta damascina* (Pisces, Cyprinidae) from the Middle East. *Italian J. Zool.*, 69:191-194.

Knibb W.R., Gorshkova G. and S. Gorshkov, 1998. Genetic improvement of cultured marine fish: case studies. pp. 111-137. In: S. De Silva (ed.). *Tropical Mariculture*. Academic Press Ltd. 487 pp.

Levan A., Fredga K. and A.A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, 52:201-220.

McKay S.J., Devlin R.H. and M.J. Smith, 1996. Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences. *Can. J. Fish. Aquat. Sci.*, 53:1165-1176.

Vladykov V.D. and C.G. Gruchy, 1972. Comments on the nomenclature of some subgenera of Salmonidae. *J. Fish. Res. Board Canada*, 2:1631-1632.