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FISH CYTOGENETICS AND ITS APPLICATION IN FISH REPRODUCTIVE BIOLOGY

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P. Rab (1991) Fish cytogenetics and its application in fish reproductive biology. *Bull. Inst. Zool., Academia Sinica Monograph* **16**: 357-373. Why fish cytogenetics (and/or cytotaxonomy) is, or would be applied in reproductive biology of fishes? Similarly as in domestic animals and birds, the progress of fish reproductive biology may profit from this discipline of cytology. Although it has developed very rapidly, especially in recent years, being in the descriptive developmental stage it is still underdeveloped in comparison with the cytogenetics of higher domestic animals. From about 20,000 fish species estimated to occur, the basic karyotype characteristics, *i.e.*, diploid chromosome number ($2n$) and number of chromosome arms (NF), are known for not more than 1,700 fish species, which represents only about 9% of the total number. Of the economically important species, these basic data are available nearly exclusively for freshwater groups such as acipenserids, salmonids, cyprinids, cichlids and several others; marine important species are cytogenetically fairly unexploited. In general, descriptions based on conventionally Giemsa stained chromosomes highly prevail; due to small chromosome size of majority of species they are also often unperfect. The application of chromosome banding techniques has been quite limited.

The future efforts in relation to the fish reproductive biology should be focused at least as follow: 1) Application and introducing novel and sophisticated chromosome banding techniques including molecular ones; 2) Establishing "standard karyotypes" for economically important species; 3) Screening for chromosomal disorders and studying their relationships to fecundity; 4) Studying pollution-related chromosomal abberation; 5) Studying sex determination at chromosomal level using molecular techniques, too; 6) Application of cytogenetic methods in fish chromosome manipulation procedures including gene transfer.

In conclusion, one can suggest that cytogenetics in fish biology should play an analogous role as it does in biology and veterinary

sciences of domestic animals offering large application potential and, thus, its contribution is unreplaceable.

Key words: Fish cytogenetics, Reproductive biology, Prospectives.

Since the research on the reproductive biology of fishes concerns on the species of economical or other (*e. g.*, artificial propagation, conservation, etc.) importance the situation with application of cytogenetic methods parallels to that in veterinary sciences. The remarkable achievements of veterinary cytogenetics (*e. g.*, Fechheimer, 1984) may, therefore, serve as a good comparative tool to our subject. For example, the application of conventional and banding karyotyping techniques to a number of different domestic animal species has provided a vast array of more of less generally accepted standard karyotypes. Karyotype characteristics as revealed by banding patterns confirmed the hypotheses regarding the origin of many domestic animals from their wild ancestors. Chromosomal polymorphisms of different kinds have been discovered and their distributions have been studied in many strains, races,, breeds and/or species of domestic animals. Cytogenetic analyses of abortuses, neonatates, malformed young or infertile individuals have demonstrated the relationships of various chromosomal abberations with etiology of such disorders. The physical and chemical agents cause chromosomal damages as has been evidenced in livestocks from polluted regions. Developments in molecular biology have provided a number of extremely useful techniques which enable a direct, cytological mapping of gene localizations.

Why fish cytogenetics (which refers to studies of various aspects of the chromosome morphology, structure and function) and/or cytotaxonomy (which utilizes some of thus obtained data to compare the chromosome sets between cell populations, individuals, populations or higher taxonomic units) is or would be applied to the reproductive biology of fishes? Similarly as in domestic animals and birds, the progress of fish reproductive biology may profit from both these disciplines of cytology. This paper is by no means exhaustive as it summarizes the thesis of a lecture presented at the Symposium; its aim is to briefly review the current status of fish

cytogenetics and to show what cytogenetic data and techniques can be utilized in the fish reproductive biology.

BRIEF REVIEW OF CURRENT STATUS OF FISH CYTOGENETICS

The first information on fish chromosomes dates back to the last decade of the XIXth century (quoted in Makino, 1951), and many other data have been subsequently gathered during next about sixty years (Makino, 1951; Nogusa, 1960). However, the rapid development took place, as in cytogenetics of all animals in general, after introducing novel cytogenetic procedures, *i. e.*, colchicine inhibition of dividing cells at metaphase stage and hypotonic pretreatment of cells for spreading the chromosomes in early sixties (*e. g.*, Roberts, 1964) as well as application and development of various banding techniques since seventies (*e. g.*, McGregor and Varley, 1983). The application of such methods was concomitant with the survey of fish tissues suitable either directly or *via* cultured cells for chromosome analyses (*e. g.*, Ivanov, 1972; Denton, 1973; Ojima, 1982; Blaxhall, 1985; Hartley and Horne, 1985; Ueda, 1986). Although the limiting factors still remain obtaining of consistently good metaphase cells with well spread chromosomes and some details concerning the application of chromosome banding analyses, the fish cytogeneticists are now armed with many powerful tools which enable their use in other fields of research activities, too.

A) Methodology

Although it is clear that the progress in any field of research activity depends primarily on the development of its methodology, the aim of this paper is not to review or discuss the methodological pecularities of fish cytogenetics. In general, the basic principles are the same as in most other animal groups, *i. e.*, obtaining and preparing chromosomes from dividing cells of different tissues either directly or *via* cell cultures. At present, direct preparing of fish chromosomes highly prevails. Despite of some minor special differences, all subsequent methods of handling with these chromosomes are basically the same as in other vertebrates. A number of extensive and good reviews on this subject exists (for most recent one, see Gold *et al.*, 1990 and

references therein) and will not be described here.

B) Fish chromosomes and karyotypes

Of about more than 20,000 fish and fish-like species estimated to occur (Cohen, 1970), the basic karyotype characteristics, *i. e.*, diploid chromosome number ($2n$) and composition of chromosome set expressed as a number of chromosome arms (NF), are known for not more than 1,700 fish species, which represents about 9% of the total fish species only. The distribution of these available data among particular fish groups and/or fish faunas is by no means irregular. Several fish groups are well cytogenetically studied in terms of number of species, populations and individuals analyzed as well as banding procedures applied while others are nearly or completely unknown. For instance, the Salmonidae as a whole are the best karyologically known fish group (Hartley, 1987) though coregonids (Jankun and Rab, 1991) or huchonines (Rab *et al.*, 1991) have been studied comparatively far less; chromosomes of salmonids have been studied extensively in North America, Europe, eastern Siberia, etc., but practically no reports exist from inner Asia (Mongolia, north-western China). The karyotypes of the family Cyprinidae, the largest fish family, are well or sufficiently well known from North America (Amemiya and Gold, 1990 and papers quoted therein), Europe (Vasiljev, 1985), eastern Asia (*e. g.* Ojima *et al.*, 1972; Lee *et al.*, 1983; Yu *et al.*, 1987), India (*e. g.* Khuda-Bukhsh *et al.*, 1986) but unknown from Sunda Archipelago and Africa, respectively (Vervoort, 1980; Rab, 1981; Oellermann and Skelton, 1990). The loaches (Cobitidae) have karyologically been analyzed in Japan and Korea (*e. g.*, Kim and Lee, 1986 and papers quoted therein), Europe (Vasiljev, 1990; Rab *et al.*, 1991) but the rich cobitid fauna of southern Asia remains nearly unknown. Sturgeons and Acipenseridae have been analyzed most of all in Europe but North American species are surprisingly unstudied (*e. g.*, Birstein and Vasiljev, 1987; Arefjev, 1987). The chromosomes of South American characins and related groups have intensively been studied (Oliveira *et al.*, 1988) but African representatives are unknown. Similar situation can be found among catfishes where some data are available for North American and Asian species but both rich founa in South America and

Africa are unexploited cytogenetically. This is also the case of many groups of cyprinodontiform fishes; there are some good studies such as for *Oryzias* (Uwa and Parenti, 1988), *Poecillia* (Sola *et al.*, 1990), but others are absent. Of the perciform groups, many data have been gathered for Centrarchidae (Roberts, 1964), Cichlidae (Oliveira *et al.*, 1988), Percidae (Rab *et al.*, 1987), Gobiidae (Webb, 1986 and references therein) and several others, but information on karyology of most other groups is far from being complete. To finish these brief review: it is evident that the freshwater fish species are far better karyologically studied than the marine ones. Of the economically or in other way important species, the karyological data are available nearly exclusively for freshwater ones such as acipenserids, salmonids, cyprinids, cichlids and several others while marine species are cytogenetically fairly unexploited. The most recent general review on chromosome numbers and brief karyotype descriptions in fishes and fish-like vertebrates can be found in Vasiljev (1985) and recently accumulated data are gathered in Chromosomal Data Retrieval List by Y. Ojima (The Japan Fish Bioscience Institute, Hyogo, Japan) or in Database of Fish Karyotypes by W. H. LeGrande (University of Wisconsin, Steven's Point, U. S. A.).

The $2n$ values in fishes range from 16 in anabatoid fish, *Sphaerichthys osphromenoides* (Calton and Denton, 1974) to about 240 in evolutionary octaploid sturgeons of the genus *Acipenser* (Birstein and Vasiljev, 1987) but range between 40 and 60 in most (about 80%) species investigated so far. The karyotypes with high chromosome numbers of about 100 and 150, respectively, occur in fish groups of polyploid origin such as acipenserids, salmonids, cyprinids, catostomids and cobitids.

No generalization can be made on karyotype structures of fishes; many groups have very similar and conserved karyotypes (*e. g.* acipenserids, leuciscine cyprinids, anguillids, poeciliids, many perciform groups, etc.) while the other have highly diversified karyotypes (*e. g.* esocoids, salmonids, cyprinodontids, oryazitids, goodeids, etc.). Chromosomes of fishes are relatively very small (given in mitotic metaphase) as compared to chromosomes of mammals or frogs though several groups (salmonids, dipnoans) have chromosomes as large or even larger as mammals. The size extent of

fish chromosomes can be well demonstrated in the karyotypes of sturgeons: their sets comprises of all sizes of chromosomes from micro-to macro-elements.

The same conclusion that generalization is impossible or at least premature, applies also to the structure of fish chromosomes. The application of banding procedures to fish chromosomes showed that methods commonly used in other vertebrates also visualize structures such as constitutive heterochromatin, nucleolus organizer regions and more or less sufficient serial bands obtained by several methods. However, the difference seems to be in producing serial bands with AT- and CG-specific fluorochromes which in fishes differentiate NOR-related heterochromatin only (Rab and Mayr, 1987 and references therein) but no or very few other bands (Mayr *et al.*, 1987). This fact may indicate the lack of AT- and CG- rich regions corresponding to G- and R-bands, respectively (Holmquist, 1988). Medrano *et al.* (1988) showed that compositional DNA compartmentalization of fish genome strictly parallels the results of such chromosome bandings and they found well expressed differences in banding properties of chromosomes among fish species of different groups. It is evident that molecular approach will undoubtedly bring many new data about the structure of fish chromosomes.

WHAT DATA AND PROCEDURES ARE APPLICABLE IN FISH REPRODUCTIVE BIOLOGY AND WHAT SHOULD BE DONE TO APPLY THEM

1) The absolute prerequiste for any application of cytogenetic data in fish biology is establishing the so-called "standard karyotype", *i.e.*, generally accepted norm to which all cytogenetic data are referred to. Such karyotype standards enable exactly to describe and list any kind of chromosome/karyotype variation, *e.g.*, abberations, abnormalities, polymorphisms, mosaicisms, sex differences, etc. Karyotype standards are available for humans (ISCN, 1978, 1985) and all domestic mammals (Ford *et al.*, 1980; ISCNDA, 1989) and are being precised continuously; *e.g.*, the Committee for Standardized Karyotype of *Sus scrofa domestica* established in 1984 at 6th European Colloquium on Cytogenetics of Domestic Animals, Zurich, Switzerland, has published the most recent standard karyotype in 1988. Among fishes, standard karyotypes are not available except for three cases.

The karyotype of European eel (*Anguilla anguilla*) has been studied to great details including chromosome banding analyses (Sola *et al.*, 1980, 1984; Wiberg, 1983; Medrano *et al.*, 1988) and karyotype standard can simply be derived from these data. The rainbow trout, *Oncorhynchus mykiss*, has cytogenetically been studied in numerous papers (for review, see Flajshans and Rab, 1990) but only Thorgaard (1976, 1977, 1983) described its chromosomes from native populations throughout the original range of distribution. Among these populations he distinguished four distinct karyotypic types to which different from domestic strains can be assigned (Flajshans and Rab, 1990). Again, the karyotype standard of this species can be derived from these data. Finally, chromosomes of the common carp, *Cyprinus carpio*, have been studied by many authors (Rab, upubl.) but nearly all descriptions of its karyotype differ each other especially due to high number ($2n=100$) of relatively very small chromosomes. Rab and Roth (1987) and Rab *et al.* (1989) proposed the idiogram and standard karyotype based on extensive metric analysis of carp chromosomes using CHROMPAC III computer program for classification of particular chromosomes (Green *et al.*, 1980, 1984). They used the wild form of common carp, *C. cyprinus haematopterus*, for the proposal of such karyotype standard to avoid the effects of inbreeding, interstrain and interpopulation crossings, domestication processes, etc. Essentially the same karyotype structure for a carp of European origin has been reported by Klinkhardt and Bunk (1988) after careful metrical analysis. In the absence of banding data other than the Ag-NOR staining, the karyotype of this species can be divided into eight more or less defined groups according to the size and morphology which were denoted A to H. It is apparent that establishing of accepted karyotype standards for other species of interest is the next necessary step and should be organized by the same way as mentioned for humans and domestic mammals.

2) By analogy with mammals and birds, the reproductive traits are undoubtedly affected negatively by various chromosomal disorders. Fish species of interest have mostly high numbers of progeny and losses which may be caused by such chromosomal defects are outnumbered by those caused by environmental factors; or, more correctly, losses caused by environmental and genetic/cytogenetic

factors, respectively, are not being distinguishable at present. While this problem may be negligible in the wild, the stocked or in by other way managed fish populations. This is apparently not the case in intensive breeding programmes with careful selection of individuals for propagation where such carriers of chromosomal defects have to be excluded from the breeding. This is an unexploited area in the fish reproductive biology. Again, the absence of standard karyotypes does not make possible to distinguish the limits between normal and abnormal karyotypes in particular karyotypes in particular species. The subsequent problem is the absence of knowledge of actual effects of such well described and defined abnormalities on the reproductive traits. At present, there are few reports only on probably chromosomal abberations in colored "koi" carps (Ojima and Takai, 1981) and some European common carp strains (Al-Sabti, 1986a, 1986b). Using the chromosome nomenclature system for common carp proposed by Rab *et al.* (1989), one of the karyotype rearrangements described by Ojima and Takai (1981) can be described (as an example) as follows: 99, −D, −F, +t (Dq, Fq), 1-4 min (*i. e*, fusion of the largest metacentrics of the group D with the largest subtelocentrics of the group F with variable number of 1-4 minute elements). The presence of minute elements in the common carp karyotype has also been recorded by several authors (Ohno *et al.*, 1967, Marian-Krasznai and Krasznai, 1978), but their origin is unclear and may represent supernumerary B-chromosomes. The fixation of such rearranged karyotypes is more probable in inbred common carp populations or lines where actual indications on lowered fecundity and survival rate in progeny exist (Kirpichnikov, 1987). Screening for chromosomal disorders and studying of their relationships to the reproductive traits is therefore another important application field of cytogenetics.

3) Fishes are firmly confined to the water environment and are therefore more exposed to pollutants released into the water than other animal groups. The part of reproductive success in fishes directly or indirectly depends on pollution-related toxicity of environment. There are three cytological methods to observe the genotoxic effects on the structure of genome. The micronuclei test (MNT) is a simple method developed originally to test

the genotoxicity of various chemical compounds in mammalian chromosomes prepared from the bone marrow (Evans, 1977). Among fishes, this method has been applied to eastern mudminnow, *Umbra pygmaea* (Hoftman and de Raat, 1982) and common carp, tench (*Tinca tinca*), and grass carp (*Ctenopharyngodon idella*) (Al-Sabti, 1986b) to test different cancerogenic and mutagenic agents. Genetic toxicology can effectively use also the other method of so-called "sister chromatid exchange" test (SCE test), a procedure being technically the same as BrdU pulse replication banding which visualizes directly the chromatid breaks and their preparation by exchanged chromatids (Perry and Wolf, 1974). The test can be utilized both *in vitro* or *in vivo* directly on living fishes according to the experimental design. The details of this test applied to fish material were given by Kligerman (1982). The increased frequencies of SCE's have been documented after exposing to many chemical mutagens in mudminnows, *Umbra* spp. (Kligerman, 1979; Kligerman and Bloom, 1976; Kligerman *et al.*, 1975, 1984; Vigfusson *et al.*, 1983), killifish, *Nothobranchius rachowi* (Kerhoff and Gaag, 1985), goodeid fish, *Ameca splendens* (Barker and Rackham, 1979), or cottid fish, *Leptocottus armatus* (Zakour *et al.*, 1984). Another way to study the physical and chemical genotoxic effects is direct observatios of abberation of chromatid (*e.g.*, breaks, gaps) or chromosomal (*e.g.* fragmentation, rings, dicentrics) types. The increased rates of such chromosomal damages have been reported after X-irradiation in mudminnow, *Umbra limi* (Mong and Berra, 1979, Suyama and Etoh, 1983), and goodeid fish, *Ameca splendens* (Woodhead, 1979); after exposition to various chemicals in common carp (Al-Sabti. 1986d), killifish, *Nothobranchius rachowi* (Hooftman, 1981), goby, *Boleophthalmus dussumieri* (Krishnaja and Rege, 1982); and simply after exposition to polluted water, too (Prein *et al.*, 1978). Technical prerequisites of suitable fish models for cytogenetic toxicology are low $2n$ value, large chromosomes, short lifespan and easy rearing and/or breeding (Kligerman, 1975, 1982; Hoeven *et al.*, 1982). However, the problem is related again to the recognition of the limits between normal and abnormal karyotypes.

4) Sex determination in fishes represents vast array of sex determining systems from purely environmental to purely genetic ones (Bull,

1983). At present, several types of heteromorphic sex chromosome systems have been described in nearly 100 species from about 1,700 karyotyped. The list of heteromorphic sex chromosomes discovered among fishes can be found in Ojima (1982, 1983), Vasiljev (1985) and Rishi (1989). Besides these heteromorphic sex chromosomes are clearly recognizable after conventional Giemsa staining, the sex chromosomes or more correctly sex-related chromosomal regions can be identified after application of the banding techniques. Haaf and Schmid (1984) described such sex-specific, C-banding and DA/DAPI fluorescence heterochromatic regions in poeciliid fish, *Poecilia sphenops*. The same system of sex-related, C-positive heterochromatin has been found in another poeciliid, *Poecilia latipinna* (Sola *et al.*, 1990). Both systems can be interpreted as sex chromosomes of the ZW/ZZ type. The reverse system of XX/XY type has been identified in the lake trout, *Salvelinus namaykush*; the sex-related heterochromatin shows visible positive quinacrine fluorescence pattern (Phillips and Ihssen, 1985). Similar system is recognizable as heterozygous; male-related, C-positive heterochromatin band localized telomerically seems to be present in the northern pike, *Esox lucius* (Rab and Roth, 1990) and probably also in other species of *Esox* (Rab, unpublished). These findings support the hypothesis that very primary step in the differentiation between X and Y (or Z and W, respectively) is the concentration of highly repetitive DNA sequence, *i. e.*, heterochromatinization in Y (or W) which precedes the morphological differentiation of heteromorphic sex chromosomes. It is very probable that such sex-related heterochromatins are much more distributed among fishes than it is known at present. The recognition of sex heterochromatins, including molecular approach with sex-specific DNA probes as done in humans and domestic mammals (Aasen and Medrano, 1990), will bring many new data about sex determination systems in fishes. Undoubtedly, this is very promising direction in fish chromosome research applicable in the reproductive biology.

5) The chromosome set manipulations in fishes are most obvious application of cytogenetics; this direction in fish genetics and reproduction may be called also "experimental and applied cytogenetics". The chromosome set manipulations refer to induced gynogenesis and

androgenesis, induction of different ploidy levels, sex reversal, interspecific hybridization connected triploidization and production of transgenic animals and have been reviewed thoroughly several times (Purodom, 1983; Thorgaard, 1983; Chourrout, 1986; Thorgaard and Allen, 1987; Rab and Linhart, 1989; Hew *et al.*, 1991; Chen *et al.*, 1991). The main problems in this field are: modes of genetic inhibition of paternal (in gynogenesis) and maternal (in androgenesis) genomes, modes of restitution of diploidy and their optimization in particular species after induction of gynogenetic and androgenetic development, evidence of non-participation of paternal (gynogenesis) of maternal (androgenesis) genomes in progeny, the estimation of inbreedig rates in gynogenetic and/or androgenetic generations, both intra- and interspecific modes of production of triploid, tetraploid or even higher-ploidy levels, administration of sex-changing agents, number and localization of gene inserts, etc. Cytogenetic techniques are or can be applied routinely in most of these problems. The success with restoration of diploidy after induction of gynogenesis (androgensis) and distinguishing of haploids, diploids or mosaic individuals can be assessed by karyological analysis. The hybrid chromosomal sets are recognizable after induction of triploidy combined with heterospecific insemination. The ploidy level in ploidy-manipulated individuals can be determined by means of direct karyological analysis, enzyme electrophoretic analysis and methods of cell/DNA content such as flow cytometry, microdensitometry, cell size measurement or Coulter Counter Channelyzer Analysis (Thorgaard, 1983; Benfey *et al.*, 1984; Wattendorff, 1986; McCarther, 1987). Simple cytological method for ploidy determination is quantification of number of nucleolar organizer regions in interphase nuclei (Phillips *et al.*, 1986); this method is rapid, inexpensive and convenient (Flajshans *et al.*, in press). Possible cytological sex determination can be confronted with results of sex reversal experiments. The introduction of *in situ* hybridization with DNA probes on fish chromosomes is necessary for localization of inserted genes to study their random and/or regular insertions. This, along with localization of other genes and constructing chromosomal maps, may also be a promising direction of research activities.

CONCLUSION

While completing this paper, two very similar reviews on the same subject, *i.e.*, fish cytogenetics and its application, and methodological improvements, respectively, appeared simultaneously (Rishi, 1989 and Gold *et al.*, 1990). This fact clearly demonstrates the need of summarizing the remarkable achievements in fish cytogenetics: it has overcome the descriptive developmental stage in terms of many techniques and procedures used and gathered data and already reached the level which enables its large and extensive utilization. It is evident that application potential of fish cytogenetics will gradually increase with introducing novel methods of chromosome analysis including molecular ones. Summarizing this brief review, one can therefore suggest that cytogenetics in the fish reproductive biology should play an analogous role as it does in the biology and veterinary science of domestic animals; it offers the large application potential and its contribution to further development of fish biology is unreplaceable.

REFERENCES

Aasen, E. and J.F. Medrano (1990) Amplification of the ZFY and ZFX genes for sex determination in humans, cattle, sheep and goat. *Biotechnology* 8: 1279–1281.

Al-Sabti, K. (1986a) Karyotypes of *Cyprinus carpio* and *Leuciscus cephalus*. *Cytobios* 47: 19–25.

Al-Sabti, K. (1986b) Comparative micronucleated erythrocyte cell induction in three cyprinids by five carcingenic-mutagenicals. *Cytobis* 47: 147–154.

Al-Sabti, K. (1986c) Chromosome complements of the goldfish (*Carassius auratus auratus*) and common carp (*Cyprinus carpio*). *Cytobios* 48: 143–150.

Al-Sabti, K. (1986d) Clastogenic effects of five carcinogenic-mutagenic chemicals on the cells of the common carp, *Cyprinus carpio* L. *Comp. Biochem. Physiol.* 85C: 5–9.

Amemiya, C.T. and J.R. Gold (1990) Chromosomal NOR phenotypes of seven species of North American Cyprinidae, with comments on cytosystematic relationships of the *Notropis volucellus* species-group, *Opsopoedus emiliae*, and the genus *Pteronotropis*. *Copeia* 1: 68–78.

Arefjev, V.A. (1989) Karyotype variablity in succesive generations after hybridization between the great sturgeon, *Huso huso* (L.), and the sterlet, *Acipenser ruthenus* L. *J. Fish Biol.* 35: 819–828.

Birstein, V.J. and V.P. Vasiljev (1987) Tetraploid-octaploid relationships and karyological evolution in the order Acipenseriformes (Pisces)-Karyotypes, nucleoli and nucleolus organizer regions in 4 acipenserid species. *Genetica* 72: 3–12.

Blaxhall, P.C. (1985) Fish chromosome techniques—a review of selected literature. *J. Fish Biol.* **7**: 315-320.

Barker, C.J. and B.D. Rackham (1979) The induction of sister exchanges in cultured fish cells (*Ameca spelendens*) by carcinogens. *Mutat. Res.* **68**: 381-387.

Benfey, T.J., A.M. Sutterlin and R.J. Thompson (1984) Use of erythrocyte measurements to identify triploid salmonids. *Can. J. Fish Aquat. Sci.* **41**: 980-984.

Bull, J.J. (1983) *Evolution of sex determining mechanisms.* The Benjamin/Cummings Publ. Corp., Menlo Pk., California.

Calton, M.S. and T.E. Denton (1974) Chromosomes of Chocolate Gourami, *Shaerichtys osphromenoides*: *A* cytogenetic Anomaly. *Science* **185**: 618-619.

Chen, T.T., R.A. Dunham and D.A. Powers (1991) Application of transgenic fish technology to aquaculture. (*this issue*)

Chourrout, D. (1986) *Genetic manipulations in fish*: *review of methods. In:* Proc. World Symp. on Selection, Hybridization, Genetic Engineering in Aquaculture of Fish and Shellfish for Consumption and Stocking. Vol. II., Berlin.

CSKSS (1988) Standard karyotype of the domestic pig. Committee for the standardized karyotype of the domestic pig. *Hereditas* **109**: 151-157.

Cohen, D.M. (1970) How many recent fishes are there? *Proc. California Acad. Sci.* **38**(Ser 4): 341-346.

Denton, T.E. (1973) *Fish Chromosome Methodology.* Charles Thomas Publ., Springfield, Illinois.

Evans, H.F. (1977) *Molecular mechanisms in the induction of chromosomal abberations. In:* Progress in Genetic Toxicology (Scott, D., B.A. Bridges and F.H. Sobels, eds.). Elsevier/North Holland, Amsterdam. pp. 57-74.

Fechheimer, N.S. (1984) Animal Cytogenetics—Perspectives and Prospects. *Proc. 6th Europ. Colloq. Cytogenet. Domest. Anim.* **1984**: 4-19.

Flajshans, M. and P. Rab (1990) Chromosome study of *Oncorhynchus mykiss* kamloops. *Aquaculture* **89**: 1-8.

Flajshans, M., P. Rab and S. Dobosz (1991) Frequency analyses of active NORs in nuclei of artificially induced triploid fishes. *Theor. Appl. Genet.* (in press)

Ford, C.E., D.L. Pollock and I. Gustavsson (eds.) (1980) Proceeding of the first international conference for standardization of banded karyotype of domestic animals. *Hereditas* **92**: 145-162.

Gold, J.R., Y.C. Li, N.C. Shipley and P.K. Powers (1990) Improved methods for working with fish chromsomes with a review of metaphase chromsome banding. *J. Fish Biol.* **37**: 563-575.

Green, D.M., J.P. Bogart and E.H. Anthony (1980) An interactive microcomputer-based karyotype analysis system for phylogenetic cytotaxonomy. *Comput. Biol. Med.* **10**: 219-227.

Green, D.M., P.Z. Myers and D.L. Reyna (1894) Chrompac III: An improved package for microcomputer-assisted analysis of karyotype *J. Hered.* **75**: 143.

Haaf, T. and M. Schmid (1984) An early stage of ZW/ZZ sex chromosome differentiation in *Poecilia sphenops* var. *melanistica* (Poeciliidae, Cyprindontiformes). *Chromosoma* **89**: 37-41.

Hartly, S.E. (1987) The chromosomes of salmonid fishes. *Biol. Rev.* **62**: 197-214.

Hartley, S.E. and M.T. Horne (1985) Cytogenetic techniques in fish genetics. *J. Fish Biol.* **26**: 575-582.

Hew, Ch., Z. Gong, S. Du, G. Fletcher and P. Davies (1991) Transgenic fish: present status and future directions. (*this issue*).

Hoeven, J. C. M. van der, I. M. Bruggeman, G. M. Alink and J. H. Koeman (1982) The killifish *Nothobranchius rachowi*, a new animal in genetic toxicology. *Mutat. Res.* **97**: 35-42.

Holmquist, G. P. (1988) *DNA sequences in G-bands and R-bands. In:* Chromosome and chromatin (Adolph, K. W., ed.) Vol. II, CRC Press, Boca Raton, Florida. pp. 76-121.

Hooftman, R. M. (1981) The induction of chromosome abberations in *Nothobranchius rachowi* (Pisces: Cyprinodontiformes) after treatment with ethylmethansulphonate or benzpyrene. *Mutat. Res.* **91**: 347-352.

Hooftman, R. N. and W. K. de Raat (1982) Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the Eastern mudminnow *Umbra pygmaea* by ethyl methanesulfonate. *Mutat. Res.* **104**: 147-152.

Ivanov, V. A. (1972) Techniques for fish chromosome preparation. *Voprosy Ichtiologii* **12**(3): 389-396 (in Russian).

ISCN (1978) An internatioal system for human cytogenetic nomenclature. *Cytogenet. Cell Genet.* **21**: 309-404.

ISCN (1988) *An international system for human cytogenetic nomenclature.* Karger, Basel, New York, pp. 1-118.

ISCNDA (1989) International System for Cytogenetic Nomenclature of Domestic Animals (D. I. Berardino, D., H. Hayes, R. Fries and S. Long eds.). *Cytogenet. Cell Genet.* **53**: 65-79.

Kerkhoff, J. F. J. van de and M. A. van der Gaag (1985) Some factors affecting optimal differential staining of sister chromatids *in vivo* in the fish *Nothobranchius rachowi. Muatat. Res.* **143**: 39-43.

Khuda-Bukhsh, A. R., T. Chanda and A. Barat (1986) *Karyomorphology and evolution in some Indian Hillstream fishes with particular reference to polyploidy in some species. In:* Indo-Pacific Fish Biology: Proc. Second Int. Conf. on Indo-Pacific Fishes (Uyeno, T., R. Arai, T. Taniuchi and K. Matsuura, eds.) Ichthyological Society of Japan, Tokyo. pp. 886-898.

Kim, I. S. and J. H. Lee (1986) A chromosomal Study on the Geneus *Cobitis* (Pisces: Cobitidae) in the Southern Part of Korea. *Bull. Korean Fish. Soc.* **19**: 257-264.

Kirpichnikov, V. S. (1987) *Genetics and Selection of Fishes.* Nauka Press, Leningrad (2nd edition).

Kligerman, A. D. (1979) Induction of sister chromatid exchanges in the central mudminnow following *in vivo* exposure to mutagenic agents. *Mutat. Res.* **64**: 205-217.

Kligerman, A. D. (1982) The Use of Cytogenetics to Study of Genotoxic Agents in Fishes. *In:* Cytogenetic Assays of Environmental Mutagens (T. C. Hsu ed.) Allanheld, Osmum. pp. 161-181.

Kligerman, A. D., S. E. Bloom and W. M. Howell (1975) *Umbra limi:* a model for the study of chromosome abberations in fishes. *Mutat. Res.* **31**: 225-233.

Kligerman, A. D. and S. E. Bloom (1976) Sister chromatid differetiation and exchanges in adult mudminnows (*Umbra limi*) after *in vivo* exposure to 5-bromo-deoxyuridine. *Chromosoma* **56**: 101-109.

Kligerman, A. D., W. E. Bishop and L. C. Valentine (1984) Use of mudminnow, *Umbra* sp., in an *in vivo* sister chromatid exchange test. *Natl. Cancer Inst. Monograph* **65**: 111-118.

Klinkhardt, M. B. and B. Buuk (1988) Die chromosomen des Karpfen (*Cyprinus carpio*). *Fischerei*

Krishnaja, A.P. and M.S. Rege (1982) Induction of chromosomal abberations in fish *Boleophthalmus dussumieri* after exposure *in vivo* to mytomycin C and heavy metals mercury, selenium and chromium. *Mutat. Res.* **99**: 71-82.

Lee, H.Y., C.H. Yu, S.K. Jeon and H.S. Lee (1983) The karyotype analysis on 29 species of freshwater fish in Korea. *Bull. Inst. Basic Sci. Inha Univ.* **4**: 79-86.

Mac Gregor, H.C. and J.M. Varley (1983) *Working with Animal Chromosomes.* John Wiley, New York.

Makino. S. (1951) *An Atlas of Chromosome Numbers in Animals.* Iowa State College Press, Ames.

Marian-Krasznai, T. and Z. Krasznai (1978) Zytologische Untersuchungen bei der Familie Cyprinidae (Pisces). *Biol. Zbl.* **97**: 205-214.

Mayr, B., M. Kalat, P. Rab and M. Lambrou (1987) Band karyotypes and specific types of heterochromatins in several species of European percid fishes (Percidae, Pisces). *Genetica* **75**: 199-206.

McCarther, N. (1987) Testing for triploids. *Freshwater Catch* (*New Zealand*) **33**: 11-12.

Medrano, L., G. Bernardi, J. Couturier, B. Dutrillaux and G. Bernardi (1988) Chromosome banding and genome compartmentalization in fishes. *Chromosoma* **96**: 178-183.

Mong, S.J. and T.M. Berra (1979) The effects in increasing dosages of X-radiation on the chromosomes of the central mudminnow, *Umbra limi* (Kirtland) (Salmoniformes, Umbridae). *J. Fish Biol.* **14**: 523-528.

Nogusa, S. (1960) A comparative study of the chromosomes in fishes with particular considerations on taxonomy and evolution. *Mem. Hyogo Univ. Agric.* **3**(1): 1-62.

Oellermann, L.K. and P.H. Skelton (1990) Hexaploidy in yellowfish species (*Barbus*, Pisces, Cyprinidae) from southern Africa. *J. Fish Biol.* **37**: 105-115.

Ohno, S., J. Muramoto, L. Christan and N.B. Atkin (1967) Diploid-tetraploid relationships among old world members of the fish family Cyprinidae. *Chromosoma* **23**: 1-9.

Ojima, Y. (1982a) Methods in fish cytogenetics. *The Nucleus* **25**(1): 1-7.

Ojima, Y. (1982b) Evolution of the sex chromosomes in Pisces. A review. *La Kromosomo* **2**(27-28): 849-859.

Ojima, Y. (1983) *Fish cytogenetics. In*: Evolution of Eukaryotic Groups (Sharma, A.K. and A. Sharma, eds.) Vol. I, CRS Press. pp. 111-145.

Ojima, Y., M. Hayashi and K. Ueno (1872) Cytogenetic studies in lower vertebrates. X. Karyotyps and DNA studis in 15 species of Japanese Cyprinidae. *Jap. J. Genet.* **47**: 431-440.

Ojima, Y. and A. Takai (1981) A karyotype study of colored-carp (*Cyprinus carpio*). *Proc. Japan Acad.* **57**(B): 7-12.

Oliveira, C., L.F. Almeida Toledo, F. Foresti, A.H. Britski and S.A. Toleds Filho (1988) Chromosome formulae of neotropical freshwater fishes. *Rev. Brasil Genet.* **11**: 577-642.

Perry, P. and S. Wolff (1974) New Giemsa method for differential staining of sister chromatids. *Nature* **251**: 156-158.

Phillips, R.B. and P.E. Ihssen (1985) Identification of sex chromosomes in lake trout (*Salvelinus namaycush*). *Cytogenet. Cell Genet.* **39**: 14-18.

Phillips, R.B., K.D. Zajicek, P.E. Ihssen and O. Johnson (1986) Application of silver staining to the identification of triploid fish cells. *Aquaculture* **54**: 312-319.

Prein, A. E., G. M. Thie, G. M. Alink, J. H. Koeman and C. L. Poels (1978) Cytogenetic changes in fish exposed to water of the river Rhine. *The Science of Total Environment* **9**: 287-291.

Purdom, C. E. (1983) Gentic engineering by the manipulation of chromosomes. *Aquculture* **33**: 1-4.

Rab, P. (1981) Karyotypes of two African barbels *Barbus bariloides* and *Barbus holotaenia*. *Fol. Zool.* **30**: 181-190.

Rab, P. and P. Roth (1987) A chromosome studies on the common carp (*Cyprinus carpio*)—proposed idiogram of carp karyotype. *Zivoc. Vyr.* **32**(10): 885-892 (in Czech).

Rab, P. and B. Mayr (1987) Chromosome banding studies in European esocoid fishes: localization of nucleolar organizer regions in *Umbra krameri* and *Esox lucius*. *Copeia* **4**: 1062-1067.

Rab, P., B. Mayr and P. Roth (1987) Karyotype study of eight species of European percid fishes (Pisces, Percidae). *Caryologia* **40**: 307-317.

Rab, P. and O. Linhart (1989) Genome manipulation in fishes: a review. *Prace VURH Vodnany* **18**: 42-52.

Rab, P., J. Pokorny and P. Roth (1989) Chromosome studies of the common carp, *Cyprinus carpio*. I. Karyotype of Amurian carp, *C. carpio haematopterus*. *Caryologia* **42**: 27-36.

Rab, P. and P. Roth (1990) Chromosome banding studies in European esocoid fishes. Identification of sex chromosomes inthe northern pike, *Esox lucius*. Abstr. 21th Int. Cytogenet. Symp., Karlovy Vary.

Rab. P. and M. Jankun (in press). *Chromosome studies of coregonine fishes: a review*. *In*: Proc. Int. Symp. on Coreqonine Biology. Quebec 1990.

Rab, P., P. Roth and E. D. Vasiljeva (1991) Chromosome banding study of the golden loach, *Sabanejewia aurata* (Cobitididae). *Japan. J. Ichthyol.* (in press).

Rab, P, M. Flajshans and P. Roth (1991) *Cytogenetics and cytotaxonomy of huchonine salmonids*. *In:* Proc. Int. Conf. on Danube salmon conservation. Zilina.

Rishi, K. K. (1989) *Current Status of Fish Cytogenetics*. *In:* Fish Genetics in India (Das and Jhingram, eds.) Today and Tomorrow's Printers and Publishers, New Delhi. pp. 1-20.

Roberts, F. L. (1964) A chromosome study of twenty species of Centrarchidae. *J. Morphol.* **115**: 401-413.

Sola, L., G. Gentili and S. Cataudella (1980) Eel chromosomes: cytotaxonomical interelationships and sex chromosomes. *Copeia* **4**: 911-913.

Sola, L., B. Camerini and S. Cataudella (1984) Cytogenetics of Atlantic eels: C- and G-banding, nucleolar organizer regions and DNA content. *Cytogenet. Cell Genet.* **38**: 206-210.

Sola, L., P. J. Monaco and E. M. Rasch (1990) Cytogenetics of bisexual/unisexual species of *Poecilia* I. C-bands, Ag-NOR polymorphisms, and sex chromosomes in three populations of *Poecilia latipinna*. *Cytogenet. Cell Genet.* **53**: 148-154.

Suyama, I. and H. Etoh (1983) X-ray-induced dicentric yields in lymphocytes of the teleost, *Umbra limi*. *Mutat. Res.* **107**: 111-118.

Thorgaad, G. H. (1976) Robertsonian polymorphism and constitutive heterochromation polymorphism in chromosomes of rainbow trout (*Salmo gairdneri*). *Cytogenet. Cell Genet.* **17**: 174-184.

Thorgaard, G. H. (1977) Heteromorphic sex chromosomes in male rainbow trout. *Science* **196**: 900-902.

Thorgaard, G. H. (1983) Chromosomal differences among rainbow trout populations. *Copeia* **3**: 650-662.

Thorgaard, G. H. (1983) *Chromosome set manipulation and sex control in fish. In:* Fish Physiology, B, Reproduction: Behaviour and Fertility Control. Vol. 9, Academic Press, Orlando. pp. 405–434.

Thorgaard, G. H. and S. K. Allen (1987) *Chromosome manipulation and markers in fishery management. In:* Population Genetics and Fishery Management. University of Washington Press, Seattle. pp. 319–331.

Ueda, T. (1986) Preliminary examination of obtaining chromosome preparation for fish breeding. *Bull. Fac. Educ. Utsunomyia* **36**: 81–85.

Uwa, H. and L. R. Parenti (1988) Morphometric and Meristic Variation in Ricefishes, genus *Oryzias:* a Comparison with Cytogenetic Data. *Japan. J. Ichthyol.* **35**: 159–166.

Vasiljev, V. P. (1985) *Evolutionary Karyology of Fishes.* Nauka Press, Moscow.

Vasiljev, V. P. (1990) Diploid-triploid complex of the loaches of the genus *Cobitis* (Pisces, Cobitidae). *Dokl. Akad. Nauk U. S. S. R.* **312**: 249–252.

Vervoort, A. (1980) Karyotype of *Cacobarbus geertsi* Boulenger (Teleostei: Cyprinidae). *The Nucleus* **23**: 76–77.

Vigfusson, N. V., E. R. Vyse, C. A. Pernsteiner and R. J. Dawson (1983) *In vivo* induction of sister-chromatid exchanges in *Umbra limi* by the insecticides endrin, clordane, diazinon and guthion. *Mutat. Res.* 118: 61–68.

Wattendorf, R. J. (1986) Rapid identification of triploid grass carp with a Coulter Counter and Channelyzer. *Prog. Fish Cult.* 48: 125–132.

Webb, C. J. (1986) Karyology of *Pomatoschistus microps* (Teleostei: Gobioidei). *J. Mar. Biol. Ass. U. K.* **66**: 259–266.

Wiberg, U. H. (1983) Sex determination in the European eel (*Anguilla anguilla* L.), A hypothesis based on cytogenetic results, correlated with findings of skewed sex ratios in eel culture ponds. *Cytogenet. Cell Genet.* **36**: 589–598.

Woodhead, D. S. (1976) *Influence of acute irradiation on induction of chromosome in cultured cells of the fish* **Ameca spelendens.** *In:* Biological and environmental effects of low-level radiation. Vol. 1. Vienna, I. A. E. A. pp. 67–76.

Yu, X., T. Zhou, K. Li, Y. Li and M. Zhou (1987) On the karyosystematics of cyprinid fishes and a summary of fish chromosome studies in China. *Genetica* **72**: 225–236.

Zakour, H. R., M. L. Landolt and R. M. Kocan (1984) *Sister chromatid exchange analysis of the coldwater marine fish, Pacific sculpin* (**Leptocottus armatus**): *a feasible system for assessing genotoxic marine pollutants. In:* Sister chromatid exchanges (Tice, R. R. and A. Hollaender eds.). Plenum Publ. Corp., New York. pp. 493–507.