

Introduction to Modern Taxonomic Tools: Karyotaxonomy

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Introduction

From about 20,000 fish species estimated to occur, the basic karyotype characteristics, i.e., diploid chromosome number ($2n$) and the number of chromosome arms (NF), are known for not more than 3,000 fish species, which represents only about 15% of the total number.

The first investigations of chromosomes were made by means of histological sections in the late nineteenth century. Then, squash techniques were performed which permitted a more accurate count of chromosome number. Later, the air-drying technique combined with colchicines and hypotonic treatment (to accumulate cells at metaphase and to ease chromosome spreading) greatly increased our knowledge not only of chromosome number, but also of chromosome morphology. Karyotyping, i.e. *paring* of the chromosomes according to their size and morphology, then became feasible. Recent development of new differential staining techniques allowed the demonstration of characteristic banding of chromosomes. These techniques have led to a better understanding of the fine characterization of individual chromosomes and to a better standardization of karyotypes.

Aquatic invertebrates have been investigated mostly with squash or air-drying techniques from tissues, while in fish, since 1960 there has been a boom in cytogenetic studies mainly due to the application of human cell culture technique. Because of this, there is more information on banding techniques in fish than in invertebrate species.

There has been a biased recording of chromosome numbers in aquatic organisms depending on the group studied. Looking at the aquatic invertebrates, some phyla are poorly investigated, e.g. the sponges or the cnidarians. With regard to minor pseudocoelomate or eucoelomate animals, published data are even more rare.

Structure of Chromosomes

Recent advances like the use of colchicines, hypotonic treatment and air-drying cell suspensions, have allowed more detailed analyses of chromosome morphology. A karyotype is characterized by the different morphological types of chromosomes, i.e. metacentric, submetacentric, subtelocentric or telocentric (sometimes called acrocentric) according to the position of the centromere. Measurement of chromosomes which give an accurate location of the position of the centromere have contributed to an important gain of knowledge, entitled beta-level karyology as opposed to alpha-level karyology in which only the number of chromosomes is reported. These new techniques have permitted interspecific comparison of karyotypes and thus the identification of chromosomal rearrangement, which are an important or significant factor in evolution.

Chromosomes are best seen during the cell division. Chromosomes are not uniform in width throughout their length, but each of them has a constriction, called as centromere. It is known to be associated with the movement of chromosomes during cell division. The terminal ends of chromosomes are called telomeres, which confer stability of their ends. The centromeres and the telomere are the constitutive heterochromatic regions.

The portions of the chromosomes on their side of the centromere are called as the chromosome arms. The total number of chromosome arms in a karyotype is

called as fundamental arm number (FN). From the arm length and position of centromere we can calculate three factors: the centromeric index, the arm ratio and the relative length of the chromosomes.

The length of the shorter arm of a chromosome

Centromeric index = $\frac{\text{Length of the shorter arm}}{\text{Total length of that chromosome}}$

This gives an idea of how much of percentage the short arm is of the whole chromosome. Based on the centromeric index, the chromosomes are classified as metacentric, sub-metacentric and telocentric.

Length of the long arm

Arm ratio = $\frac{\text{Length of the long arm}}{\text{Length of the short arm}}$

Arm ratio is always greater than or equal to one and is an alternative method to centromeric index for describing the position of the centromere and the relative lengths of two arms.

Relative length = $\frac{\text{Length of a chromosome}}{\text{Total length of all the chromosomes in the haploid set}} \times 100$

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The relative length gives us an indication of the proportion of the whole genome of the organism that is represented by the particular chromosome. However; this is a linear measurement and not a measure of mass and volume.

Chromosome Classification by Centromeric Position

Type	Centrometric position	Symbol	Centrometric index range
Metacentric	Nearly median	m	46-49
Submetacentric	Submedian	sm	26-49
Acrocentric	Subterminal	st	15-30
Telocentric	Terminal	t	<15

The chromosomes of a species can be arranged in order of size (by counting from the microscopic photograph or camera lucida drawing). The complete set of chromosomes arranged according to their size is called as karyotypes. Karyotype of different species differ in size, shape, and number of chromosomes. It is, thus a phenotypic character similar to other morphological traits like body size. Therefore, it is believed that the karyotypes in different species have evolved through natural selection. Within a species, the males and females may have minor dissimilarity in the karyotypes, generally in a pair of chromosomes are very much similar are called as autosomes.

Cytotaxonomy of Finfishes

Karyotype analysis is the basic of cytogenetics. It involves the study of chromosome number, size and morphology at the metaphase stage. Karyotypes comprise a heritable character of each species for which it is useful in taxonomic differentiations between species. This is called as Cytotaxonomy. Karyotypes variation occurs in different evolutionary lineages due to chromosomal rearrangement, which should be useful in inferring the evolutionary inter-relationship between certain taxa. This is called as cytosystematics.

Fish cytogenetics has made its real beginning with the adoption of colchicines-citrate-air dry method developed by the mammalian cytogenetics in the 1960s, which replaced the slow and cumbersome techniques of chromosome study by tissue sectioning. The karyotypes of about 10% of the total ichthyofauna are available so far in the literature. This has enriched the discipline of ichthyology particularly on the cytotaxonomic and cytosystematics aspects. The chromosome analysis is also useful in ploidy determination, species and hybrid identification and genotoxicity study of the pollutants.

Cytological Techniques

This part deals with various cytological techniques used to study the chromosomes.

Chromosome Preparation

Chromosomes can be prepared from two sources: i) in vivo- from the actively dividing tissues like kidney, spleen or gill and ii) in vitro- from the cultured cells. The in vivo method requires colchicines treatment of the fishes 2-3 hours before chromosome preparation. Colchicine prevents the polymerization of tubuline that form microtubules. As a result, cell division is arrested at metaphase stage. The number of metaphase plates obtained depends up on mitotic activity, which is affected by the season, health and age of the individual fish. Prior treatment of a mitogen such as phytohaemagglutinin, cobalt chloride, phenyl hydrazine, which stimulates cell diviosns, is useful in obtaining greater number of metaphase plates.

A good number of metaphase plates can be obtained by short term in vitro culture of blood cells, abdominal fluid, fin tip or scale epithelium. The cultured cells are treated with colchicines to three hours before harvest. Long term culture not recommended for karyotypes study because aneuploid and polyploid cells are often produced.

The cells obtained from in vivo or in vitro sources are treated with a hypotonic solution, which is 0.56% potassium chloride or 1% sodium citrate. As a result the cells swell in size. The cells are sedimented by low speed centrifugation and

fixed in methanol: acetic acid (3:1 V/V) with two to three changes. The chromosomes slides are prepared by flame drying or air drying method.

Staining of Chromosomes

The slides are stained in 4-5% Giemsa solution for the visualization of chromosomes. The karyotypic analysis in fishes is inherently difficult due to the presence of a large number of small-sized chromosomes. In many fishes the karyotypes are symmetrical, that is, the chromosomes are not much different in size and morphology. For example, the karyotypes in two species of tilapia consist of 22 pairs of chromosomes. Out of these, 20 pairs are very much similar in size and morphology and the remaining two pairs only look different. Thus it is difficult to discriminate these related species having identical chromosomes unambiguously. The development of chromosome banding techniques is a further advancement in the methodology that rendered the analysis of the chromosome morphology and structure with enhanced ability.

Chromosome Banding Techniques

Chromosome bands are produced due to the variations in longitudinal structure of the chromatids, revealed by various techniques. Q-banding is named after quinacrine mustard, which is a flurochrome that stains the chromosome differentially. C-band stains deeply the centromeric heterochromatic region

present in the centromeric zone of a chromosome. Serial bands (popularly called as G- and R- bands) could be seen throughout the chromosomes by treating them with hot saline solution and staining with Giemsa. Techniques are available to demonstrate nuclear organizer region (NOR), where 18S and 28S rRNA genes are clustered. In last few years the radiolabelled DNA probes are replaced by fluorescent labelled DNA probes for Fluorescent in situ hybridization (FISH) study. This is useful in detecting the chromosomal location of a DNA sequence.

Nor Banding

Nuclear organizer region on the chromosomes can be detected by Silver staining the metaphase chromosomes. Silver staining of NOR band is relatively a simple method. The mechanism of silver staining is not fully known yet but it is believed that silver stain binds to the non-histone proteins of the transcriptionally active NOR region. The number and location of NOR bands are useful for systematic purpose. In some species it is found that the number of NOR bands is related to ploidy level. For example, in certain species belonging to the Order *Acipenseriformes*, the increase in ploidy is also accompanied by increase in the number of NOR bands. *Acipenser ruthenus* ($2n=118+4$) possesses two pairs of chromosomes. But in the related species *A. baceri* ($2n=250+8$) and *A. naccani* ($2n=246+8$), four pairs of NOR bands are present, which suggest that these species are tetraploid. However, this is not universal. In some species like *Salvelinus* NOR bands are found in multiple chromosome location.

Interestingly, sex-specific differences in NOR location was observed on the metaphase chromosomes of Mosquito fish, *Aplocheilichthys panchax*.

C-Banding

C- band can be demonstrated by

- i. BSG (barium hydroxide/saline/Giemsa) technique developed by Sumner (1972) is regarded as the standard C-banding method. Slides are hydrolysed by 0.2N HCl, denatured in barium hydroxide solution, treated with 2×SSC (saline sodium citrate) and stained with Giemsa.
- ii. By staining the metaphase chromosome with restriction enzymes, C- band can also be demonstrated.

Information on C-banding is available in over 100 species of fishes. C-banding in fishes includes not only the centromeric region but often it demonstrates heterochromatin at the telomeric and inter-calary region. In some cases the whole arm is found to be C-band positive.

Fluorescence in Situ Hybridization(FISH)

The radiolabelled DNA probe for in situ hybridization has been replaced by fluorescent dye-labelled DNA probe in the recent past. The fluorescence signal is detected by the fluorescent microscope and various colour patterns on

chromosome can be obtained. This technique is now used for homologous chromosome identification and studying the location of a DNA sequence on the chromosome. In the near future greater use of this technique would be found in fish cytotaxonomy and genetic studies.

Fish Chromosomes and Karyotypes

Fish chromosomes and karyotypes of the economically important species karyotype data are available nearly exclusively for freshwater groups such as acipenserids, salmonids, cyprinids, cichlids and several others: marine important species are cytogenetically unexploited.

The $2n$ values in fishes range from 16 in anabantoid fish, *Sphaerichthys osphromenoides* to about 240 in *Acipenser* but range between 40 and 60 in most (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 karyotypes (e.g. acipenserids, anguillids, poeciliids, many perciform group, etc) while others have highly diversified karyotypes (e.g. salmonids, cyprinodontids, etc). Chromosomes of fishes are relatively very small as compared to chromosomes of mammals or frogs through several groups (salmonids diploans) have chromosomes as large or even larger as mammals.

Trends in Karyotypes Evolution

Chromosome evolution in fishes can be traced back to the protochordates. The tunicates, considered to be a primitive group among protochordates, contain microchromosomes only. A microchromosome is generally less than 0.5 micrometer (μm) in size, whereas average size of fish chromosomes range between 2 to 5 μm . It is believed that large chromosomes have arisen by increase of DNA content through polyploidy and fusion of microchromosomes. It is interesting to note that the lungfish, *Lepidosiren paradoxa* possess very large chromosomes of 15 to 30 μm in length.

In the cartilaginous fishes – sharks, skates and rays, the diploid number of chromosomes varies between 28 to 106, with most species having chromosomes between 52 to 100. The chromosomes are often present.

In the chondrostein fishes of the families *Polyodonidae* and *Acipenseridae*, the chromosome numbers and genome sizes are very large. Microchromosomes are present in these species. It is believed that fishes of these families are polyploid of various degrees i.e tetraploid to octaploidy. The karyotypes of the tetraploid species (*Acipenser huso*, $2n=118+2$ and *Polyodon spathula* , $2n=120$) could be divided into groups of four chromosomes of similar morphology. The holosteian fishes, *Lepisosteus oculatus*) has chromosome number $2n=68$ and *Amia calva* has $2n=46$, which includes some microchromosomes. Holostei are believed

to be the closest relatives of the *Acipenseriform* fishes but the chromosome number in the former is comparatively low. This point also suggests that *Acipenseriform* fishes originated by polyploidy.

The teleostean fishes show a wide variation in chromosome number but they lack microchromosomes. In the order *Salmoniformes*, the chromosome numbers of the family *Salmonidae* is comparatively higher than the members of the related families. The members of this family are believed to have originated by polyploidy. Among *Cypriniformes*, all species of the family *Catostomidae* and some members of the families *Cyprinidae* and *Cobitidae* are polyploids. Of about 450 *cyprinidae* species studied, it is remarkable to find majority of them having 50 chromosomal complements in their karyotypes. The variations of chromosome number is more pronounced in the order *Siluriformes*. Polyploid is found in the family *Callichthyidae* of this order. On the other hand, the chromosome number is less variable in the order *Perciformes*, where most species possess $2n=48$ chromosomes.

Many fish cytogeneticists believe that 48 acrocentric chromosomes constitute the basic diploid karyotypes in teleosts. The karyotypic diversity in fishes arose by polyploid, Robertsonian fission/fusion and other chromosomal rearrangements like pericentric inversion. Chromosomal non-disjunction (i.e. unequal separation of two chromosome sets into two daughter cells) during meiosis produces of chromosomal polymorphism within a species. Robertsonian fission/fusion takes

place when the chromosome number changes without any corresponding change in the fundamental arm number (FN). For example, if two acrocentric chromosomes get fused to form a metacentric chromosome or a metacentric chromosome gets split into two acrocentric chromosomes, occurs no change in the FN of chromosome arms. There are three interesting points to be noted in the study of fish karyotypic evolution. i) The presence of polyploid forms in some orders. ii) The chromosomes are distinguishable in only a few species. iii) Intra-specific chromosomal polymorphism occurs in some species.

Polyploidy In Fishes

Polyploid forms are found naturally in six different orders of fishes: *Acipenseriformes*, *Salmoniformes*, *Cypriniformes*, *Siluriformes*, *Poecilloformes* and in one species of *Perciformes*. Tetraploid fishes are, however, the most common among the polyploid forms. The yellowfish species of the genus *Barbus* (*Cyprinidae*) found in South Africa are hexaploids. The *Acipensiform* species *Acipenser gueldenstaedti* is an octaploid species having the chromosome number, $2n=250+8$.

The polyploid origin of *Salmo salar* (*Salmonidae*) is suggested on the basis of the presence of quadrivalents in the meiotic prophase, duplicate expression of isozyme genes and for possessing high C-value. *Cyprinus carpio* (*Cyprinidae*) is also believed to be a tetraploid species based on its high chromosome number and C-value although quadrivalent formation does not take place during meiosis. Some isozyme loci manifest tetraploid expression pattern but a good number of genes in this species are expressed in diploid dosage. Thus, although the genome is polyploid, many gene loci behave as diploid in terms of gene expression. This has led to the belief that *C. carpio* is in the process of diploidization.

Polyploidy is believed to have played an important role in the evolution of fishes. Allopolyploidy, which is formed by polyploidization of the hybrids of related species, might have played more important role than the autopolyploidy. The triploid *poeciliid* fishes probably originated by allopolyploidy.

Sex Chromosomes in Fishes

At present, several types of heteromorphic sex chromosome have been described in nearly 100 species from about 1,700 karyotyped.

Almost all types of sex chromosomal mechanisms such as male heterogamety, female heterogamety and multiple sex chromosomes are observed in fishes. This is quite expected since they display almost all types of sexuality found among the vertebrates. Male heterogamety, that is XX-XY sex chromosomes, is more prevalent among fishes than the female heterogamety (ZZ-ZW). Male heterogamety, for example, is found in some deepwater fishes and female heterogamety in several species of catfishes. The occurrence of multiple sex chromosomes was reported in few species like *Chiondraco harmatus*, an Antarctic fish. The sex chromosomes in lake trout (*Salvelinus namaycush*) could be detected by banding techniques. The X and Y chromosomes in this species are distinguishable by the prominent C-band on the X-chromosomes, which is much reduced in case of the Y-chromosomes. The recognition of sex heterochromatins, including molecular approach with sex-specific DNA probes as done in humans and domestic mammals, will bring many new data about sex determination systems in fishes.

Diploid Chromosome

The diploid chromosome number of some commercially important species are given below

Sl.No.	Species	Chromosome No.
1.	<i>Gonostoma bathyphylum</i>	12
2.	<i>Salmo salar</i>	58
3.	<i>Carassius auratus</i>	104
4.	<i>Cyprinus carpio</i>	104
5.	<i>Channa striatus</i>	40
6.	<i>C. gachua</i>	78
7.	<i>C. punctatus</i>	16
8.	Catla, Rohu, Mrigal	50
9.	<i>Oreochromis mossambicus</i>	44
10.	<i>O. niloticus</i>	44
11.	<i>Heteropneustes fossilis</i>	58
12.	<i>Clarias batrachus</i>	50

13.	Grass carp	48
14.	Silver carp	48
15.	<i>Penaeus monodon</i>	88