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Karyomorphology of two major carps, Catla catla and Labeo rohita

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Abstract

A detailed study of chromosomes of two species of Indian inland fish revealed the chromosomal number and type. It describes the relation between the two species. Their peculiar morphological characters have been recorded. These karyological observations provide strong evidence to conclude that the two species are closely related and have phylogenetic links.

Introduction

Cytogenetics is the study of chromosome morphology and the behaviour of chromosomes during mitosis and meiosis. The chromosome number and its morphology is specific for a particular species. Fishes are of particular interest to Ichthyologists as well as cytogenetists as they occupy a very important position in the systematic differentiation of vertebrates. The benefits of this Karyological study among fishes are great values as fishes are economically important.

Karyological methods of ascertaining the taxonomic position of different species of fish have been in wide use in recent years among Russian and other ichthyologists (E. A. Salmenkova et al, 2005). The available data in fishes show that almost all forms of chromosomal rearrangements have played a role in the evolution of the fish karyotypes (E.D.Vasil'eva 2011).

Materials and Methods

Experimental species

Four different species of freshwater fish namely *Catla catla* Hamilton (Catla); *Labeo rohita* (Hamilton-Buchanan (Rohu);) were selected for the present study.

Maintainence in the laboratory:

The live animals were collected from Poondi fresh water aquaculture station and they were transported to the laboratory in oxygenated polythene bags and maintained in fiber glass tanks containing enough amount of water. The water was changed once a day and the fishes were given food adlibitum. The fishes were acclimatizes for about one week before the experiments were conducted. Feeding was stopped two days prior to the experiment. On the thirds day, the experimental fishes were injected with colchicine and introduced into the tank.

Chemicals required

- Colchicine 0.01% (10mg colchicine was dissolved in100ml of dis. water.
- Potassium chloride:0.4%,400mg KCl was dissolved in 100ml of dis. water.
- Sodium citrate:0.9% 900mg of sodium citrate was dissolved in 100ml of dis. water.
- Carnoy's fixative: 3:1 ratio of methanol and Glacial acetic acid

Giemsa Stain

Prepared by dissolving 2ml of stock Giemsa solution and 2ml of 10% disodium hydrogen phosphate to 4.6ml of distilled water (PH 6.8).

Karyotyping

Procedure developed by Kligerman 1982 was followed with minor alterations.

Since blood samples could not be obtained in smaller fishes, chromosome preparation from the gill tissues were used for

chromosome preparation.

Staining

The air dried slides were then stained with freshly prepared Giemsa staining solution (4%) for 15-18mts. The slides were then destained with distilled water and air dried. The slides were then screened for chromosomal spreads under the light microscope.

Chromosomal complement in*Catla catla*:

The total diploid number was found to be 50(2n=50). This was confirmed by observing 157 metaphase plates which showed the diploid number 50.

Based on the idogram individual karyomorphology of the diploid set was analysed and the chromosome length was measured. The length ranges from 8.5X10-3µ to 3.0 X10-3µ of the largest to the smallest chromosomes. In the diploid set four pairs are metacentric (4,9,15 and 20), Thirteen pairs are submetacentric (1,2,3,5,6,8,10,11,12,14,16,19 and 21) and remaining eight pairs are acrocentric (7,13,17,18,22,23,24 and 25). Relative length percent (RL%) ranges from 5.64 to 1.99. Nucleolar organiser region (NOR) and heterochromatin region (HCR) were also observed. (Fig 1, Table 1 and Plate 1 & 2).

Fig. 1: –

An Idiogram of*Catla catla*.

Pair No	Pair means p(µ)	q(µ)	TL(µ)	RL%	Ic	TYPE OF CHROMOSOME
1	$2X10^{-3}$	$6.0X10^{-3}$	$8.3X10^{-3}$	5.64	0.24	Submetacentric
2	$1.5x10^{-3}$	$6.0x10^{-3}$	$8.0x10^{-3}$	5.31	0.19	Submetacentric
3	$2.0x10^{-3}$	$4.5x10^{-3}$	$7.0x10^{-3}$	4.65	0.29	Submetacentric
4	$3.0x10^{-3}$	$3.5x10^{-3}$	$7.0x10^{-3}$	4.65	0.43	Metacentric
5	$2.5x10^{-3}$	$4.5x10^{-3}$	$7.0x10^{-3}$	4.65	0.36	Submetacentric
6	$1.5x10^{-3}$	$4.75x10^{-3}$	$6.75x10^{-3}$	4.48	0.22	Submetacentric
7	$0.5x10^{-3}$	$5.5x10^{-3}$	$6.5x10^{-3}$	4.31	0.08	Acrocentric
8	$2.0x10^{-3}$	$4.0x10^{-3}$	$6.5x10^{-3}$	4.31	0.31	Submetacentric
9	$3.0x10^{-3}$	$3.0x10^{-3}$	$6.5x10^{-3}$	4.31	0.46	Metacentric
10	$2.0x10^{-3}$	$4.0x10^{-3}$	$6.5x10^{-3}$	4.31	0.31	Submetacentric
11	$1.75x10^{-3}$	$4.1x10^{-3}$	$6.3x10^{-3}$	4.22	0.28	Submetacentric
12	$1.5x10^{-3}$	$4.25x10^{-3}$	$6.25x10^{-3}$	4.15	0.24	Submetacentric
13	$0.75x10^{-3}$	$5.0x10^{-3}$	$6.25x10^{-3}$	4.125	0.12	Acrocentric
14	$1.75x10^{-3}$	$4.0x10^{-3}$	$6.25x10^{-3}$	4.15	0.28	Submetacentric
15	$2.75x10^{-3}$	$2.75x10^{-3}$	$6.0x10^{-3}$	3.98	0.46	Metacentric
16	$2.0x10^{-3}$	$3.5x10^{-3}$	$6.0x10^{-3}$	3.98	0.33	Submetacentric
17	$0.5x10^{-3}$	$4.8x10^{-3}$	$5.8x10^{-3}$	3.85	0.09	Acrocentric
18	$1.0x10^{-3}$	$4.25x10^{-3}$	$5.75x10^{-3}$	3.82	0.17	Acrocentric
19	$1.75x10^{-3}$	$3.5x10^{-3}$	$5.75x10^{-3}$	3.82	0.30	Submetacentric
20	$2.5x10^{-3}$	$2.5x10^{-3}$	$5.5x10^{-3}$	3.65	0.45	Metacentric
21	$2.0x10^{-3}$	$3.0x10^{-3}$	$5.5x10^{-3}$	3.65	0.36	Submetacentric
22	$1.0x10^{-2}$	$4.0x10^{-3}$	$5.5x10^{-3}$	3.65	0.18	Acrocentric
23	$0.5x10^{-2}$	$2.5x10^{-3}$	$3.5x10^{-3}$	2.32	0.14	Acrocentric
24	$0.5x10^{-3}$	$2.0x10^{-3}$	$3.0x10^{-3}$	1.99	0.17	Acrocentric
25	$0.5x10^{-2}$	$2.0x10^{-3}$	$3.0x10^{-3}$	1.99	0.17	Acrocentric

p : Short arm
q : Long arm
TL : Total length
RL % : Relative length percent
Ic : Centromere index

Table 1:

Measurement of chromosomal complement of*Catla catla*.

Plate 1:

Catla catla.

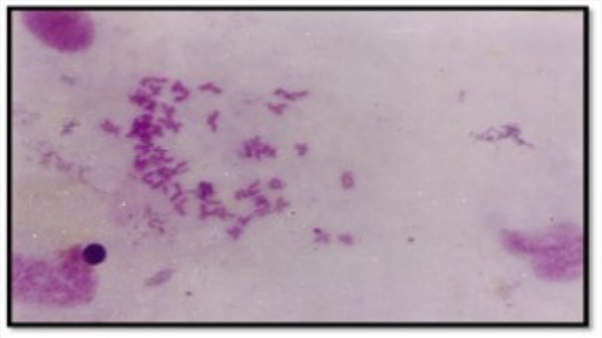


Plate 2:

Metaphase plate showing chromosomal complement 2n = 50 of *Catla catla*.

Chromosomal complement in Labeo rohita

The diploid number was found to be 50(2n=50). This was confirmed by observing 133 metaphase plates which showed the diploid number 50.

The chromosomes are condensed in nature and darkly stained with distinct karyomorphology. Based on the idogram individual karyomorphology of the diploid set was analysed and the chromosome length was measured. The length ranges from 6.5X10-3µ to 2.2 X10-3µ of the largest to the smallest chromosomes. In the diploid set eleven pairs are metacentric (2,3,4,5,10,12,14,15,20,23 and 24), Remaining fourteen pairs are submetacentric (1,6,7,8,9,11,13,16,17,18,19,21,22 and 25) and remaining eight pairs are acrocentric (7,13,17,18,22,23,24 and 25). Relative length percent (RL%) ranges from 6.43 to 2.18. Nucleolar organiser region (NOR) was found in pair one and heterochromatin region (HCR) were observed in all the other pairs. (Fig 2, Table 2 and Plate 3 & 4).

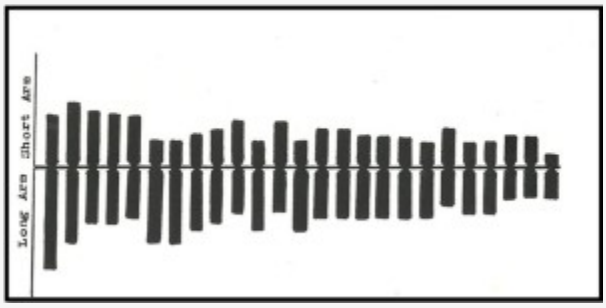
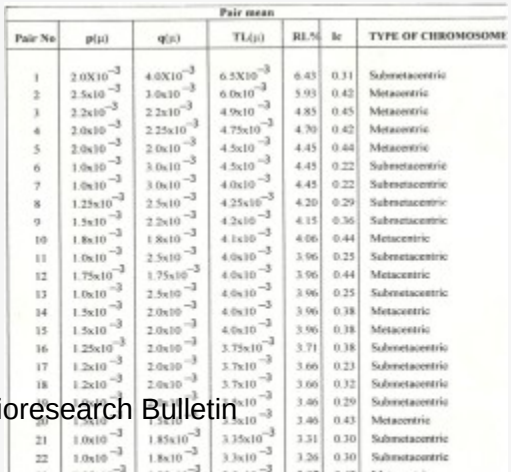


Fig. 2: –

An Idiogram of *Labeo rohita*.



Pair mean						
Pair No	p(µ)	q(µ)	TL(µ)	RL%	Ic	TYPE OF CHROMOSOME
1	$2.0X10^{-3}$	$4.0X10^{-3}$	$6.5X10^{-3}$	6.43	0.31	Submetacentric
2	$2.5x10^{-3}$	$3.0x10^{-3}$	$6.0x10^{-3}$	5.93	0.42	Metacentric
3	$2.2x10^{-3}$	$2.2x10^{-3}$	$4.9x10^{-3}$	4.85	0.45	Metacentric
4	$2.0x10^{-3}$	$2.25x10^{-3}$	$4.75x10^{-3}$	4.70	0.42	Metacentric
5	$2.0x10^{-3}$	$2.0x10^{-3}$	$4.5x10^{-3}$	4.45	0.44	Metacentric
6	$1.0x10^{-3}$	$3.0x10^{-3}$	$4.5x10^{-3}$	4.45	0.22	Submetacentric
7	$1.0x10^{-3}$	$3.0x10^{-3}$	$4.0x10^{-3}$	4.45	0.22	Submetacentric
8	$1.25x10^{-3}$	$2.5x10^{-3}$	$4.25x10^{-3}$	4.20	0.29	Submetacentric
9	$1.5x10^{-3}$	$2.2x10^{-3}$	$4.2x10^{-3}$	4.15	0.36	Submetacentric
10	$1.8x10^{-3}$	$1.8x10^{-3}$	$4.1x10^{-3}$	4.06	0.44	Metacentric
11	$1.0x10^{-3}$	$2.5x10^{-3}$	$4.0x10^{-3}$	3.96	0.25	Submetacentric
12	$1.75x10^{-3}$	$1.75x10^{-3}$	$4.0x10^{-3}$	3.96	0.44	Metacentric
13	$1.0x10^{-3}$	$2.5x10^{-3}$	$4.0x10^{-3}$	3.96	0.25	Submetacentric
14	$1.5x10^{-3}$	$2.0x10^{-3}$	$4.0x10^{-3}$	3.96	0.38	Metacentric
15	$1.5x10^{-3}$	$2.0x10^{-3}$	$4.0x10^{-3}$	3.96	0.38	Metacentric
16	$1.25x10^{-3}$	$2.0x10^{-3}$	$3.75x10^{-3}$	3.71	0.38	Submetacentric
17	$1.2x10^{-3}$	$2.0x10^{-3}$	$3.7x10^{-3}$	3.66	0.23	Submetacentric
18	$1.2x10^{-3}$	$2.0x10^{-3}$	$3.7x10^{-3}$	3.66	0.32	Submetacentric
[illegible]	[illegible]	[illegible]	[illegible]	3.46	0.29	Submetacentric
[illegible]	[illegible]	[illegible]	[illegible]	3.46	0.43	Metacentric
21	$1.0x10^{-3}$	$1.85x10^{-3}$	$3.35x10^{-3}$	3.31	0.30	Submetacentric
22	$1.0x10^{-3}$	$1.8x10^{-3}$	$3.3x10^{-3}$	3.26	0.30	Submetacentric

Table 2:

Measurement of chromosomal complement of *Labeo rohita*.

Plate 3:

Karyotype of *Catla catla*.

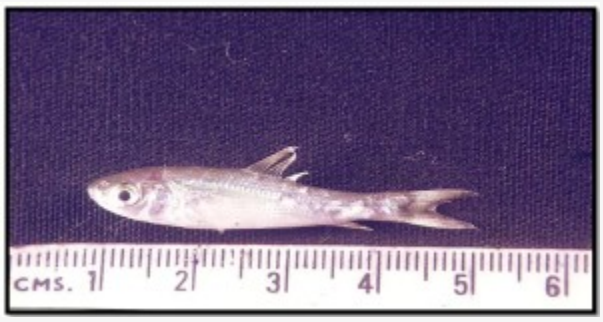


Plate 4:

Labeo rohita

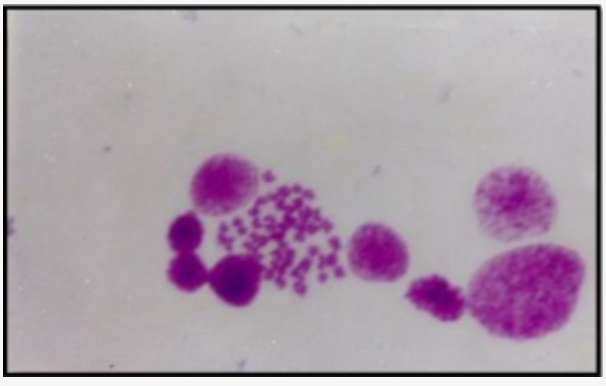


Plate 5:

Metaphase plate showing chromosomal complement 2n = 50 of *Labeo rohita.*

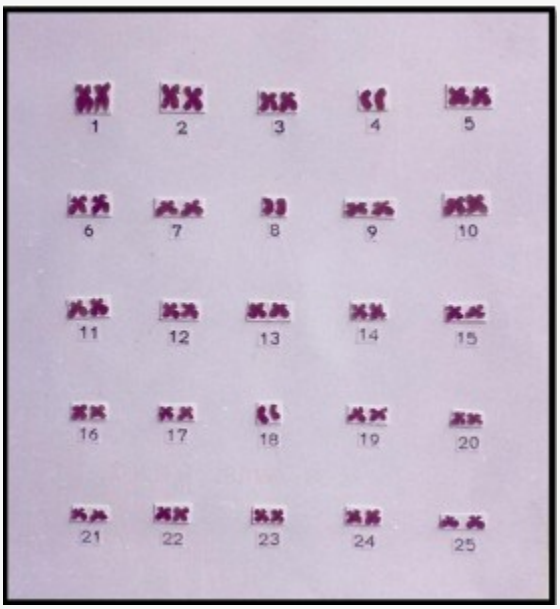


Plate 6:

Karyotype of *Labeo rohita*

Discussion

Diversity is a land mark of evolution. Each species is characterized by a specific chromosome complement commonly referred to as karyotype. Karyodiversity is mainly due to the variation in the position of centromere. Centromere is the special region of chromosome which gets firmly attached to mitotic spindle at the time of metaphase during cell cycle. These are usually observed during the early stage of cell division as non-staining gaps. The chromatids, both attached to the kinetochore part of centromere and to the spindle during metaphase which can be observed by the influence of a drug, colchicines. Besides the centromere, non-stained gaps in the form of secondary contriction is also a common feature of chromosomes.

During the early and late metaphase, the chromosomes reach maximum degree of condensation and contriction, to take part in the on-going chromosomal division. Hence, metaphase holds the secret of cell cycle and expresses the full complement of chromosomes in the somatic cells of a species is referred to as somatic number and designated as 2n. In the present study gill cells, conventional karyological technique of Kligerman and Bloom (1977) was adopted with due modification to suit the experimental fish. In the present study the diploid complement 2n was 50 in both the species of carps.

The number of chromosomes per cell is rather a conservation characteristic and may be used as an indicator of closeness of species, within families. The number and position of the arms of the chromosomes is even more conservative than chromosome number and is often equally useful in taxonomic studies.

Conclusion

In conclusion it is emphasized that the order Cypriniformes include fish groups which is not much variable from karyological point of view. But to analyse in detail the chromosome evolution processes and taxonomical relationship, it is necessary to collect more cytological banding methods.

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