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Note

Estimation of genome size in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton), *Cirrhinus mrigala* (Hamilton) and *Labeo calbasu* (Hamilton) by Feulgen microdensitometry method

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ABSTRACT

Genome size of Indian major carps *viz*., rohu, catla, mrigal and kalbasu has been reported here by estimating nuclear DNA content of Feulgen-stained blood smears in microdensitometer. The Nile tilapia (*Oreochromis niloticus*) genome size was taken as the internal reference standard. Although diploid chromosome number in all these species are same (2n =50), they show notable difference in their genome size *viz*., 1.044 pg (kalbasu), 1.047 pg (mrigal), 1.994 pg (rohu) and 2.447 pg (catla). These differences may be attributed to the content of junk DNA and degree of developmental complexity of the species. The study is first of its kind in Indian carps and the information would help in generating linkage maps for identification of trait associated genes.

Keywords : Indian major carps, Genome size, Microdensitometry

The genome of an organism contains its whole hereditary information encoded in the DNA (RNA in some viruses). This includes both the coding and non-coding sequences of the DNA. Genome size in eukaryotes has typically been expressed as the mass (in pictogram= pg) of DNA per haploid nucleus (Gregory, 2005). The C-value, a term coined in 1950 by Swift in reference to the haploid class of DNA content, is now used interchangeably with 'genome size' in diploid animals (Gregory, 2001). In fish, genome size appears to be directly related to the rate and complexity of development (Hardie *et al*., 2002). Study of genome size is important from the stand point of 'C-value paradox' of genome structure and evolution. Besides, it has more practical utility in genome mapping and sequencing programmes. It gives an instant approximation of the amount of non-coding DNA present in a given genome.

Several methods have been employed to estimate nuclear DNA of plants and animals, which include biochemical quantification, reassociation kinetics, Feulgen-stained microdensitometry and flow cytometry techniques. Feulgen microdensitometry offers a rapid, cost effective as well as user friendly method of genome estimation of a huge number of taxa. Fish genome size quantification, in particular, has been used by Feulgen-stained blood smears followed by microdensitometry analysis (Hinegardner and Rosen, 1972; Majumdar and McAndrew, 1986; Carvalho *et al*., 1998) or blood cell samples stained with base-specific fluorochromes and analyzed by flow cytometry (Thorgaard *et al*., 1982; Stefano *et al*., 2005).

The Indian major carps, catla, rohu, mrigal and kalbasu are fast growing and highly preferred food fishes in India. These carps have also gained popularity in other South-east Asian countries. So far, there is no report on the genome size of the Indian major carps. Current research program in our laboratory aims at generating a low density linkage map by using genetic markers in one of these species. The genome size information would help gather knowledge on the number of markers necessary for generating a map with a specific resolution in one of these carp species for identification of trait genes. Therefore, genome sizes of Indian major carps have been studied using densitometry method in the present investigation.

Live specimens were collected from the fish farm of the Institute. Due care was taken during their identification to avoid any inclusion of hybrid genotype. Three male individuals of same age group (~700 g) from each species were considered. Blood was collected from heart and smeared on the frosted end of glass slides. After air drying, samples were hydrolyzed in 1 N HCL for 15 min at 60 ºC, rinsed with distilled water twice to wash excessive acid and then incubated in 45% acetic acid (15 min) for swelling of nuclei. The time of hydrolysis was determined empirically. Then the slides were stained in Schiff's reagent for two and half hours at room temperature. To prevent

over-oxidation of the dye, gelatin films were put to cover the slides and kept in dark. The Nile tilapia was used as the reference standard (1.08 pg/nucleus, Kocher, 2005) for estimation of the DNA content. For hydrolysis, ten slides were made from each species and stained but only three slides from each were studied. Slides were washed (15 min in each step) in butanol and alcohol with various ratios (1:3; 1:1; 3:1; 1:0; 1:0 butanol and alcohol respectively). The DNA was estimated using a microdensitometer (Nikon Optiphot, Japan), setting the monochromatic light at 550 nm and following the method of Sharma and Sharma (1980). From each slide, 30 well separated and rounded nuclei were considered for estimation with the aperture at 1, under a 100X oil-immersion objective. To express the DNA amount in picogram (pg), the transmittance (T) value was converted to optical density (O.D.) and DNA estimated from the reference standard. The length of genomes in terms of nucleotide base pairs was obtained from the conversion of picogram to base pairs (Dolezel *et al*., 2003).

Haploid DNA content of the species ranged from 1.044 pg (kalbasu) to 2.447 pg (catla). The results are presented in Table 1. Though all the species possessed a 2n = 50 chromosomes (Zhang and Reddy, 1991), catla genome size was found to be two fold higher than kalbasu genome and the variation could be due to differences in non-coding region (junk DNA) (Dolezel *et al.*, 2003). Overall analysis of the data showed a mean value of 1.633 pg, close to 2.0 pg of overall fish genomes as reported by Hinegardner and Rosen (1972). However, our data differ significantly from the mean genome size (2.85 pg) of family Cyprinidae (Hardie *et al*., 2004). This could be due to the fact that some cyprinids such as common carp and mahseer are natural polyploids and polyploids can have a larger genome size.

Table 1. Chromosome numbers, haploid DNA content (standard deviation=SD) and nucleotide bases (million base =Mb) of Indian major carps

Species name	Chromo-some numbers (2n)	Haploid DNA content (pg/ nucleus) (SD).	Approximate nucleotide base pair number
Labeo rohita	50	1.994 (0.164)	1950Mb
Labeo calbasu	50	1.044 (0.062)	1021Mb
Catla catla	50	2.447 (0.164)	2393Mb
Cirrhinus mrigala	50	1.047 (0.218)	1023Mb

The main objective of this study was to gather preliminary knowledge on genome size of Indian major carps, particularly rohu for which a gene mapping program is in progress. Prior information on size of the genome is essential in order to make an estimate of number of DNA markers that will be necessary to generate a genetic map with specific resolution for application such as identification of trait associated genes. It is estimated that catla, rohu, mrigal and kalbasu having genome size of 2393, 1950, 1023 and 1021 Mb of DNA, respectively may require at least 200 markers each in rohu and catla and 100 each in the rest to produce 10 cM resolution map.

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