J. Ecophysiol. Occup. Hlth. 10 (2010) 121-127

Cytogenetic characterization of B_1R and BC_1F_2 backcross progenies of *Catla catla* (Ham.) and *Labeo rohita* (Ham.)

S. K. Tripathy, K K Gaur[1] and N. Sarangi[2]

Trident School of Biotech Sciences, Chandrasekharpur, Bhubaneswar, 751024, Orissa, India
[1]Department of Zoology, RBS College, Agra, 282002, UP, India
[2]Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar, 751002, Orissa, India,

Abstract :*The diploid chromosome number (2n) and the chromosomal morphologies of the backcross progenies of Catla catla (Ham.) and Labeo rohita (Ham.) viz.-B_1R and BC_1F_2 developed in Central Agricultural Research Institute, Port Blair, South Andaman, India are reported for the first time through this study. The B_1R backcross generations includes carps developed by hybridization of B_1 backcross female and rohu male. The B_1 backcross individuals were developed by taking F_1 hybrid females from cross of catla female and rohu male hybridized with parental catla male. Similarly, the BC_1F_2 backcross was developed by inter se breeding of B_1 backcross progenies. The conventional Giemsa-Flame drying technique of karyotyping provided first hand information regarding the cytogenetic character of the above two generations of backcross progenies. It was found that, the diploidy in both generations was 50, with variation in chromosomal morphometry i.e.- 2n=50: 14M + 10 SM + 10 ST + 16 T/A in B_1R and 2n= 50: 8M +12 SM + 22 ST + 8 T/A. in BC_1F_2.*

Key Words: *Catla catla*, *Labeo rohita*, Backcross, Diploidy, Karyomorphology

Introduction

The two Indian major carps *viz.- Catla catla* (Ham.) and *Labeo rohita* (Ham.) are commonly known as catla and rohu, are most imperative for freshwater aquaculture in Indian peninsula (Jhingran and Pullin, 1985) and are among the world's principal aquaculture species in terms of production (Hulata, 2001). Genetic evaluation of a species/variety through karyotyping serves as a prologue for its identity which is further augmented with various banding techniques. Karyotypes in general are employed for comparison among different groups of organisms. It is very useful in cases to distinguish experimental inter-specific races in actual hybridization, which are not easily recognizable by external morphology alone. The present investigation employs the chromosomal data for identification of backcross progenies of catla and rohu i.e. B_1R and BC_1F_2. It provides the first hand information regarding the diploidy (2n) in the above two backcross generations of catla and rohu.

Materials and Methods

The parental generations viz.- catla (P_1) and rohu (P_2) were developed from the seeds procured by the Fisheries Science Division of Central Agricultural Research Institute (CARI), Port Blair, South Andaman from the carp breeding unit of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar as a part of regular breeding programme (Sarangi, 1998 and Sarangi *et al.*, 2002). The F_1 hybrids were developed by successful inter-generic hybridization between catla female and rohu male in the indoor carp hatchery unit of CARI following the standard procedures to induce breeding through hypophysation. The progenies of generation developed from crossing F_1

female and parental catla male were designated as B_1 where as those from the female B_1 backcross and male parental rohu were designated B_1R. Those developed by the *inter se* breeding of B_1 backcross progenies were designated as BC_1F_2. Chromosomal investigations through karyotyping were carried out for the B_1R and BC_1F_2 backcross progenies. That for the parental generations viz. - catla, rohu, and F_1 hybrids was not required as a lot of references exist in literature.

The backcross progenies were maintained in separate aquariums and plastic pools with short term rearing facilities having round the clock aeration and water flow through system. A total of 15 (fifteen) specimens from B_1R and 17 (seventeen) from BC_1F_2 backcrosses were used for the purpose. The fishes were subjected to acclimatization in laboratory condition after immediate procurement from the culture pond with facility of continuous aeration and feeding *ad libitum*. After acclimatization, the individuals were subjected to starvation in separate aquarium one by one. Starved individuals marked by fin cutting was injected by mitogen i.e.- Concanavalin-A (Con-A) or Phytohemagluttinin PHA (1mg/ml in Phosphate Buffer Saline PBS) @ 1ml/100 g body weight with the help of 1.0 ml insulin glass syringe of 18-20 gauze needle size. The experimental animals were administered with the first dose prior to 48 hrs of actual sacrifice and with second dose 24 hrs prior to sacrifice. The individuals were injected with metaphase arrestor i.e.-colchicine @ 1ml/100 g body weights just 2-3 hrs before dissection and collection of kidney tissues. Conventional method for the metaphase spread preparation with flame drying was adopted following Kligermann and Bloom (1977). Staining was done on the next day of each slide preparation by immersing in 4% Giemsa working solution (BDH) for one and half hours to two hours insidse a Couplin jar. Finally, the slides were de-stained by showing to free flow of de-ionized water. These were air dried for 30-45 minutes and screened under low magnification.

A total of 63 slides from B_1R and 71 slides from BC_1F_2 backcross progenies were prepared and screened mounting to total of 138 and 177 metaphase spreads from respective backcross progenies under low magnification (10X). Some selected spreads were re-screened and comparatively better plates were magnified to 100 X under oil emersion. Photographs were taken for analysis with the help of attached photographic accessories of the OLYMPUS binocular research microscope. The enlarged prints of the plates were developed. Direct counting of chromosome numbers was carried out manually from each photographic plate followed by assignment of karyomorphologies to each set of chromosome as per Levan *et al.* (1964) manually.

Results and Discussion

Photographs of one representative spread from each of both backcross generation (Fig. 1 and 2), comparative Fig. (3 and 4) and the Tables (1 to 4) present the findings for comparison, compilation and convenience of understanding.

The chromosome number in both B_1R and BC_1F_2 backcross progenies were found same as those in parental carps viz. catla and rohu ($2n = 50$). The modal diploid chromosome numbers of 50 was found in 95.65 % of observation in B_1R backcross progenies and 97.74 % of observations in BC_1F_2 backcross progenies. The numbers of metacentric, submetacentric, subtelocentric and telocentric/ acrocentric chromosomes in both B_1R and BC_1F_2 were 14 and 8; 10 and 12; 10 and 22 as well as 16 and 8 respectively. Hence the diploid formulae were found to be 2n=50 (14M+10 SM+10ST+16T/A) in B_1R backcross progenies and that of BC_1F_2 backcrosses was 2n= 50 (8M+12SM+22ST+8T/A).

Table 1. Modal diploid chromosome numbers (2n) of B_1R and BC_1F_2 backcross progenies of catla and rohu

No.	Backcross	No. of individuals	No. of metaphase	Diploidy	
				2n	**No of spreads**
1	B_1R	15	138	48	01
				49	01
				50	132
				51	04
				Modal diploidy (%)	**95.65**
2	BC_1F_2	17	177		
				49	02
				50	173
				51	01
				52	01
				Modal diploidy (%)	**97.74**

Table 2. Deviation of diploid chromosome number (2n) of B_1R and BC_1F_2 backcross progenies of catla and rohu from the parental diploidy

No.	Backcross	Observations	Control	Replications	Observations/replication	Mean (Std. dev.)
1	B_1R	138	50	3	46	50 .0 ± 0.365 50.0 ± 0.210 50.021± 0.147
2	BC_1F_2	177	50	3	59	49.983± 0.130 50 .0 ± 0.185 50.034± 0.260

Table 3. Karyomorphologies of B_1R and BC_1F_2 backcross progenies of catla and rohu with diploid number 50

No.	Backcross	Observations	No. of spreads	Distribution of various type of chromosomes		
				Type	No.	No. of spreads
1	B_1R	132	12	M	14	12
				SM	10	10
				ST	10	12
				T/A	16	10
2	BC_1F_2	173	10			
				M	08	10
				SM	12	09
				ST	22	10
				T/A	08	10

The chromosomal morphologies (Levan *et al*, 1964) still remains the backbone of karyotype study though improvisation of cytological techniques. As per Levitzky (1931), asymmetric karyotypes with large differences in smallest and largest chromosomes of the set having fewer metacentric pair is considered relatively advanced in comparison to symmetric type. Similarities in them are presumed to represent evolutionary kinship. Chromosome studies in fishes be it karyotype or banding, lags behind as compared to most other vertebrates attributed to the small size and large number of chromosomes (Banerjee, 1987; Gold *et al*, 1990). As per Rishi (1989), diploidy in fishes ranges from very small as 12 in *Gonostoma*

Table 4. Comparative karyomorphologies of B_1R and BC_1F_2 backcrosses progenies with parental catla, rohu and F_1 hybrids

	Chromosomal morphologies						References
	2n	**FN**	**M**	**SM**	**ST**	**T/A**	
Catla	50	-	6	32	-	12	Majumdar and Ray-Chaudhuri (1976)
Catla	50	78	8	16	14	4 + 8	Manna and Khuda-Buksh (1977 a and b)
Catla	50	-	8	16	14	12	Manna (1983)
Catla	50	78	12	16	-	22	Zhang and Reddy (1991)
Catla	50	78	12	16	-	22	Jana (1993)
Rohu	50	-	6	26	18	-	Majumdar and Ray-Chaudhuri (1976)
Rohu	50	78	12	8	8	22	Krishnaraja and Rege (1979)
Rohu	50	-	18	8	4	20	Manna (1983)
Rohu	50	78	10	18	22	-	Zhang and Reddy (1991)
Rohu	50	78	10	18	22	-	Jana (1993)
Rohu	50	-	6	16	8	20	Nagpure (1997)
F_1	50	-	4	24	8	14	Lakra and Rishi (1991)
F_1	50	88	12	10	16	12	Jana (1993)
B_1R	50	-	14	10	10	16	*
BC_1F_2	50	-	8	12	22	8	*

2n: Diploid number; FN: Fundamental number; M: Metacentric; Sm: Sub-metacentric; St: Sub-telocentric; T/A: Telocentric/Acrocentric

* As per the present findings

bathyphylum or 16 in *Sphaerichthys ospheromonoides* to a large number of 239±7 in *Acipenser naccari.* Majority of families showed a peak value of 2n = 48 (520 species) followed by 2n = 50 (238 species) and 2n = 46 (138 species). Distribution of diploid number in fishes was reported largely leptokurtic with 70% species having range of 2n = 44-52 and about 80% lying in the range of 2n = 40-56 (Rishi, 1989). Variation in the chromosome number and karyotype in the level of intra-individual; intra and inter-population was an established fact (Kirpichnikov, 1981). As per Campos *et al.* (1997), cypriniformes are more primitive than siluroids as the ratio of sub-telocentric to telocentric chromosomes is more than the ratio of metacentric to sub-metacentric in their karyotypes.

Karyomorphology studies of the parental carps by earlier reports showed very inconsistent findings. This inconsistency may be due to technical limitations or possible polymorphism available in these two carps in respect to the geographic localities (Barat and Sahoo, 2001). The probable reason for controversies on karyomorphologies in parental carps may be due to large number of small chromosomes, which caused observational variations in accurate determination by different workers. This is imminent as arbitrary localization of centromeric position in the small bi-armed chromosomes with out help of banding technique often leads to some degree of variation in karyotypic description. As all the earlier references on karyotypes of catla, rohu and F_1 agree unanimously on the same diploid number (2n) i.e.-50, it may be due to the reason that, both the carps are different at generic level, still they have high chromosomal compatibility leading to successful hybridization (Zhang and Reddy, 1991) and maintaining the same diploidy making their progenies fertile for the next generation in contrast to other sterile hybrids in plants and animals. This may be a probable

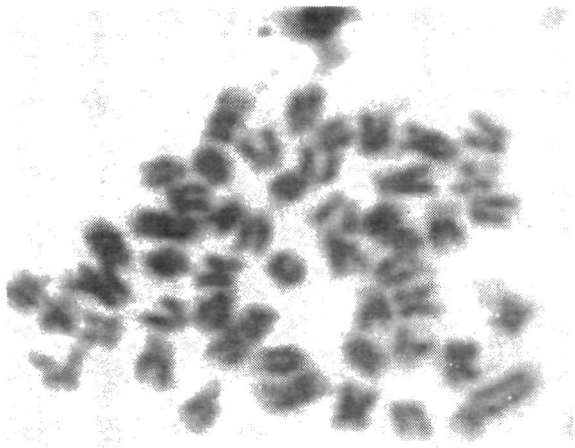

Fig. 1. Metaphase spread of B_1R, 2n=50: (14M+10 SM+10ST+16T/A)

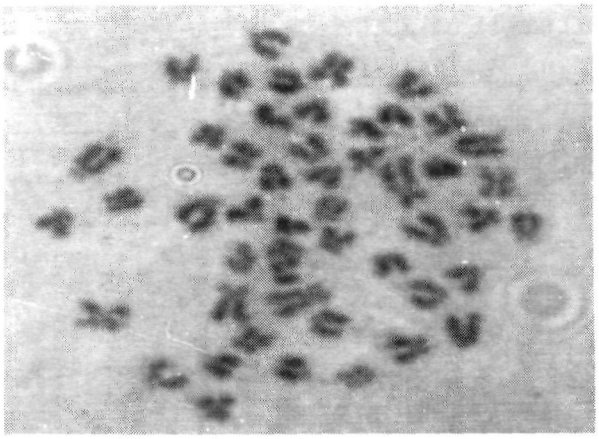

Fig. 2. Metaphase spread of BC_1F_2, 2n= 50: (8M+12SM+22ST+8T/A)

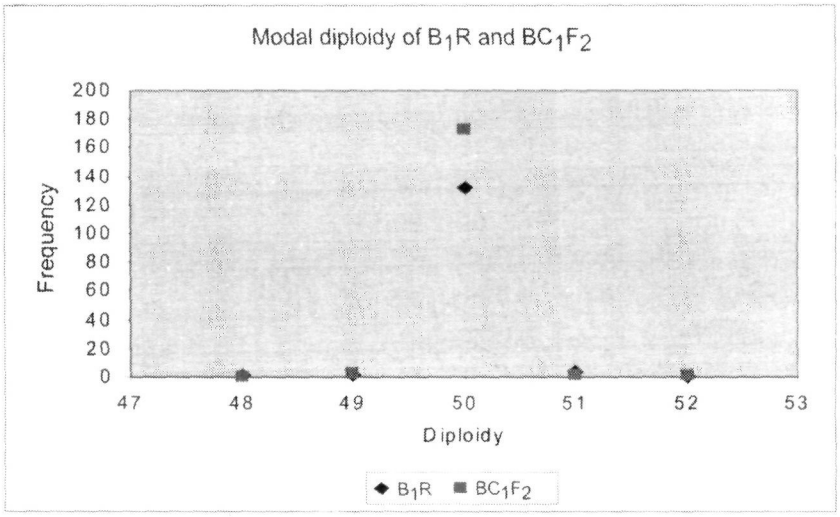

Fig. 3. Schematic diagram showing highest diploid number in B_1R and BC_1F_2 backcross progenies of catla and rohu

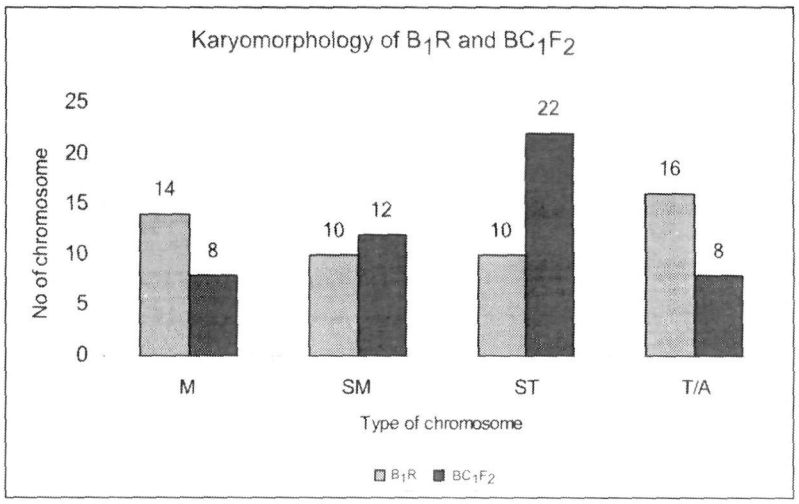

Fig. 4. A comparison of karyomorphologies in backcross generations

explanation for successful backcrossing in catla and rohu. Tripathy and Sarangi (2009) presented some generalized expects of catla – rohu hybridization with description of chromosomal morphometries.

The typical diploid chromosome numbers of both parents were reported to be 50 by various earlier authors (Table 4). The results of present findings in the same table is supported by plates 1 and 2 showing representative metaphase spreads from B_1R and BC_1F_2 backcross generations. The progenies confirm the same number of chromosome as in parental catla, rohu and F_1. This indicates that, chromosomal compatibility has been maintained in further backcross generations of catla and rohu beyond F_1. As per Reddy and Tantia (1992) the differences in karyomorphology of grasscarps reported earlier by various workers agree on numbers only but contrast each other on the morphologies which might be due to over-exposure to colchicine causing excessive contraction of chromosomes obscuring the small arm of small metacentric chromosomes and giving them a false appearance of acrocentric chromosomes. Hence such variations in karyomprphologies contrasting each other reports may not be due to chromosomal polymorphism and it may be the case in karyotypes of various Indian major carps. The karyotype of B_1R and BC_1F_2 backcross progenies in the present findings shows 2n= 50 in both cases with differences in their karyomorphologies. The number of metacentric chromosomes (14) of B_1R is exactly intermediate to both the parents and (8) of BC_1F_2 is closer to rohu. Number of sub-metacentric chromosomes of B_1R (10) and BC_1F_2 (12) are intermediate to parental catla and rohu but more similar to rohu. Number of sub-telocentric chromosomes of B_1R (10) similar to that of catla and BC_1F_2 (22) is more alike rohu. Number of telocentric/acrocentric chromosomes of B_1R (16) is similar to rohu and that of BC_1F_2 (8) is closer to catla (Majumdar and Ray Chaudhuri, 1976). However, this finding is insufficient to pinpoint a definite trend of inheritance from their parental generations which requires support from other tangible techniques of present day cytogenetics e.g. C-banding, R banding, Q banding or fluorescent *in situ* hybridization (FISH) to get higher resolution for better transparency on the architecture of chromosomal pattern in various generation of catla and rohu developed *enroute* backcrossing.

Acknowledgement

The authors highly acknowledge the facilities provided by the then Director, Central Agricultural Research Institute, Port Blair for carrying out the study successfully and the A P Cess fund Agency under Indian Council of Agricultural Research, New Delhi, India for sponsoring the external funded projects to Fisheries Science Division, Central Agricultural Research Institute, Port Blair, South Andaman, India during the financial year 1999-2002.

References

Banerjee, S., (1987) Induction of fish mitotic division *in vivo* by concanavalin-A. *Ind. J. Exptl. Biol.*, **25,** 556-558.

Barat, A and Sahoo, P. (2001) Chromosomal polymorphism in fish. *Persp. Cytol. Genet.*, G. K. Manna and S. C. Roy (eds.), **10,** 591-598.

Campos, H., Arratia, G. and Cuevas, C. (1997) Karyotypes of the most primitive catfishes (Teleostei: Siluriformes: Diplomystidae). *J. Zool. Systemat. Evol. Res.*, **35,** 113-119.

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

Hulata, G. (2001)Genetic manipulation in aquaculture: a review of stock improvement by classical and modern technologies. *Genetics*, **111,** 155-173.

Jana, R. K. (1993) Studies on some aspects of biology and cytogenetics of rohu-catla and catla-rohu hybrids. Thesis submitted for the degree of Doctor of Philosophy (Science) of the University of Kolkata, 50-60.

Jhingran, V. G. and Pullin, R. S. V. (1985) A hatchery manual for the common, Chinese and Indian major carps. ICLARM studies and Reviews 11, Asian Development Bank, Manilla, Philipines and ICLARM, Manilla, Philipines, pp.191.

Kligermann, A. D. and Bloom, S. E. (1977) Rapid chromosome preparation from solid tissues of fishes. *J. Fish. Res. Board Can.*, **34,** 266-269.

Kirpichnikov, V. S. (1981) *Genetic bases for fish selection.* Springer-Verlarg, Berlin, New York, pp. 410.

Krishnaraja, A. P. and Rege, M. S. (1979) Genetic studies on two species of the Indian carps *Labeo* and their fertile F1 and F2 hybrids. *Ind. J. Exptl. Biol.*, March 1979, **17,** 253-257.

Lakra, W.S. and Rishi, K. K.(1991) Chromosomes of Indian fishes an annotated list. *Ind. J. Anim. Sci.*, **61,** 342-349.

Levitzky, G. A.(1931) The karyotype in systematics. *Bull. Appl. Bot. Genet. Plant Breed.*, **27,** 220-240.

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

Majumdar, K. and Chaudhuri Ray S. P.(1976) Studies on the chromosomes of Indian major carps. *Proc. Symp. Modern Trends Zool. Res. India*, Calcutta, 68-69.

Manna, G. K. and Khuda-Buksh, A. R. (1977) a Karyomorphology of cyprinid fishes and cytological evolution of the family. *The Nucleus*, **20,** 119-127.

Manna, G. K. and Khuda-Buksh, A. R. (1977) b A checklist of chromosomes in cyprinid fishes. *J. Zool. Res.*, **1,** 34-43.

Manna, G. K. (1983) Cytogenetic studies on fishes and Amphibia. *Genet. Res. India*, (ICAR): 244-273.

Nagpure, N. S. (1997) Distribution of C-band heterochromatin in *Labeo rohita (Ham.)* Cyprinidae. *Chromos. Sci.*, **1,** 45-46.

Reddy, P. V. G. K. and Tantia, M. S.(1992)Recent advances in fish chromosome methodology. *J. Aquacult.*, **2,** 17-20.

Rishi, K. K. (1989) Current Status of Fish Cytogenetics in India. In: *Fish Genetics in India*, P. Das & A.G.Jhingran (eds), New Delhi, 1-20.

Sarangi, N. (1998) Carp hybridization and performance of hybrids in fresh water of islands. *Proc. Symp. Island Ecosys. Sust. Development*, Organized by Andaman Science Association and Dept of Science and Technology 116-120.

Sarangi, N.,Tripathy, S. K., Mandal, A. B. and Ahalawat, S. P. S. (2002) Studies on some genetical trends in backcross population of Indian Major Carps viz. *Catla catla* and *Labeo rohita*. In: *Proc. Fifth Ind. Fish. Forum*, held in Central Institute of Freshwater Aquaculture, Bhubaneswar, 105-110.

Tripathy S. K. and Sarangi, N (2009) An overview on Genetics and Hybridization of *Catla catla* (Ham.) and *Labeo rohita* (Ham.) in India. *Emerging Science* in Ed. by G.C. Swain.

Zhang, S. M. and Reddy, P. V. G. K. (1991) On the comparative karyomorphology of three Indian Major Carps *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.). *Aquacult.*, **97,** 7-12.