academicJournals

Vol. 12(36), pp. 5503-5512, 4 September, 2013
DOI: 10.5897/AJB2012.2943
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Morphotypes vis-a-vis genetic parameters of *Catla catla* (Ham.) and *Labeo rohita* (Ham.) backcrosses

S. K. Tripathy[1]*, K. K. Gaur[2] and N. Sarangi[3]

[1]MITS school of Biotechnology, Plot No. 2P, Chandaka Industrial Estate, Infocity Square, PO- KIIT campus, Patia, Bhubaneswar-751024, Odisha, India.
[2]Department of Zoology, R. B. S. College, Dr. B. R. Ambedkar University, Agra, 282002, Uttar Pradesh, India.
[3]Central Institute of Freshwater Aquaculture, Kaushalyaganga, Bhubaneswar, 751002, Odisha, India.

Accepted 21 August, 2013

Backcross generations of *Catla catla* (Ham.) and *Labeo rohita* (Ham.) were developed in Central Agricultural Research Institute, Port Blair, South Andaman, India, using the technique of induced breeding for Indian Major Carps. The trend of morphometry through generation mean analysis indicates reduction of head size with respect to standard length, which is considered as a reduction of bone size within whole body biomass. The segregation pattern of dominant head morphometries of rohu and partial dominance of body morphometries of catla was supported by subsequent genetic evaluation through karyotyping, biochemical analysis and PCR-random amplified polymorphic DNA (RAPD) based molecular marker analysis indicating more genetic proximity of rohu with backcrosses than catla. The present study is significant for carp genetics with special reference to catla and rohu.

Key words: Backcross, catla, esterase, karyomorphology, molecular marker, morphometries, rohu.

INTRODUCTION

The two Indian major carps namely:- *Catla catla* (Ham.) and *Labeo rohita* (Ham.) are scattered naturally in various river systems of India, Pakistan, Burma and Bangladesh (Jhingran and Pullin, 1985). They are among the world's principal aquaculture species in terms of production (Hulata, 2001) and differ in many ways with respect to their phenotypic traits. As per the study of Basavaraju et al. (1995), larger head per unit body weight of catla is considered as a major disadvantage for freshwater aquaculture when the edible flesh content per unit body mass is concerned. Therefore, a good amalgamation of deep catla type body and narrow rohu type head is always a notion of considerable importance for aquaculture requiring apt hybridization.

Keeping this in view, the present study was undertaken to develop different backcross generations of catla and rohu with an aim to develop a new variety/strain with certain desirable traits. The desirable traits with aquaculture importance include narrower head, broader, longer and thicker body in percentage of total length and stouter caudal peduncle keeping in view of the percentage of edible flesh content in comparison with the total body weight. Success to achieve this target could be anticipated through selective breeding to make narrower and longer head, as well as, broader and thicker body by introgression of responsible gene(s). In similar genetic improvement programme, disease resistance, field performance and breeding capacity etc are also other parameters of concern but, those were not taken in to consideration in this study.

The major objective in the present study was to get different generations of Indian major carps namely:- *Catla catla* and *Labeo rohita* through systematic breeding and as it is very difficult to get parental generations, F1 and

*Corresponding author. E-mail: sbskt04@rediffmail.com/sbskt04@yahoo.co.in. Tel: +91-9439535406

F2 hybrids, as well as, various backcross generation at a time due to their long generation period of 2 to 3 years. At the same time genetic analysis through evaluation of the developed progenies was done involving generation mean analysis (GMA) of morphometric parameters to get a clear trend followed by comparative karyotyping; esterase profiling and PCR-RAPD based molecular markers analysis.

Comparative karyotyping was essential to find any major change in the genome at chromosomal level as chromosomes are the simplest indicators of change in genome at cellular level through change in ploidy or any other change in the number of each type of chromosome, that is, metacentric, sub-metacentric, telocentric, sub-telocentric anatenctric, acrocentric, etc. The technique of biochemical genetics through isozyme analysis and PCR-RAPD marker based analysis was adopted to find the correlation between genetic distances/similarities between various generations of carps with respect to segregation of phenotypic trends from parents to progenies. This was desired to find genetic proximity of catla or rohu parents with other successive generations indicating the contribution towards development of longer and narrower head like rohu and broader, deeper and thicker body like catla.

MATERIALS AND METHODS

Developing the breeds of backcross generations

For the above purpose, the base stocks of parental generations were developed from the seeds of *Catla catla* (Ham.) and *Labeo rohita* (Ham.) in farm facilities of Central Agricultural Research Institute (CARI), Port Blair, Andaman, India (Tripathy et al., 2010) from the founder stocks of the parental generations developed from the seeds procured from the hatchery unit of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha during 1987 as a part of hatchery development programme in CARI. During subsequent years, pure stocks of catla and rohu were developed through regular breeding programme of the institute to meet the demand of local farmers each year. Side by side, various tissue samples were collected and preserved for use in future; as well as, morphomtric measurements were documented for analysis. The mating design mostly followed was 1:1 brooders for each experimental analysis purpose whereas it was 2:3 or 1:3 (male:female) for farm requirement purpose. The breeds were maintained in separate pools and ponds without allowing any mix up.

The F_1 hybrids were developed by crossing catla and rohu and were designated as C×R or CR. Subsequently, the F_2 hybrids were produced from *inter se* breeding of F_1 progenies (CR×CR), the first backcross generation or B_1 was produced from F_1 and catla (CR×C), whereas B_2 from F_1 and rohu (CR×R). Hybridization of B_1 and rohu resulted in B_1R (CR×C)×R and BC_1F_2 were from *inter se* breeding of B_1 (CR×C) × (CR×C). A long and tedious process of breeding, hatchery management and maintaining the breeds in isolated ponds was undertaken from 1987 to 2008 to set the objective of backcross breeding and genetic evaluation taking the help of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha, India and CARI from time to time. However the detail of induce breeding for the period of F_1, F_2 and backcross development is presented in Table 4.

Morphometry and generation mean analysis (GMA)

All metrical values of the morphometric parameters were converted to percentage of standard length and mean percentage values of each generation along with their standard error of means as analyzed Balon (1995). For the Generation Mean Analysis (GMA), three replication means of each morphometric ratio of all generations with corresponding values of variance and variance of means were used by GENRES. For this study, 73 catla, 50 rohu, 36 F_1 hybrids, 30 B_1 backcross, 30 F_2 hybrids, 30 B_2 backcross, 30 B_1R and 30 BC_1F_2 backcross were measured for various morphomteric parameters.

The age group of catla and rohu ranged from 1 to 3 years while those of F_1 and B_1 were between 6 months to 2 years. For the rest other generations, the age group varied between 6 months to 1 year. Various morphometric parameters included mean percentage values of head, trunk and tail length as well as their depth and thickness in percentage of standard length running from tip of the snout to the base of the caudal fin. For measuring each morphemetric parameter, replica of three set individuals were collected during harvest/sampling consisting of 25 to 30 each for catla and rohu. Those of other generations consist of 10 to 15 per sampling. The morphometric analysis mostly included mean percentage values of nine important parameters in percentage of standard length for length, depth and thickness of head, body and caudal peduncle; as well as, those of snout length, eye diameter and pre-dorsal, pre-pectoral, pre-anal and pre-pelvic length. The detail of morphometric parameters recorded is presented in Table 5.

The generation mean analysis was performed using the statistical software GENRES (1994) by putting the mean percentage values of morphometric parameter like length, depth and thickness of head and body for comparison. The data in Tables 1 and 2 present different scaled values as per scaling scaling and six parameter test, respectively and different inter-genetic interactions resulting in form of various epistatic phenomena, that is, duplicate or complementary epistasis. The scaling scaling test used means, variance and means of variance giving result in form of scaled values such as A, B, C and D scales with their corresponding variances and t values as per Haymann and Mather (1955). In these classical genetic model like scaling scaling test and six parameter model the co-efficient of variations which are the percentage values of standard deviations (Falconer, 1981; Mather and Jinks, 1982; Phanse and Sukhatme, 1995; Falconer and Mackay, 1996) are employed to get the final out put in form of all the four scales (A, B, C and D) and the genetic interactions values like M, D, H, I, j and L.

The scaled value like A is calculated by the software based on statistical formula, which is $A= 2B_1-P_1-F_1$ and variance of A (V_A)= $4V(B_1) + V(P_1)+ V(F_1)$ where B_1 indicates the mean values of the character of the 1st backcross generation, P_1 presents that of 1st parent (catla) and F_1 presents that of 1st filial progenies. The corresponding values of variances for each generation are represented by V (X_n). Similarly, the scaled value $B = 2B_2-P_2-F_1$ and $VB= 4V(B_2) +V(P_2) +V(F_1)$. The scaled value $C= 4F_2-2F_1-P_1-P_2$ and $Vc = 16 V(F_2) + 4V(F_1) + V(P_1) + V(P_2)$ and $D = 2F_2- B_1-B_2$ and $VD= 4V(F_2) + V(B_1) + V(B_2)$. The values of standard error for each scale is calculated by taking the square root of corresponding variances and t values mentioned in Table 1 are the proportion of each scale with respect to their standard errors having an exclusion limit of 1.96 at P =0.05.

The Table 2 presents another classical genetic test; that is, six parameter model of GMA to reveal non-interacting crosses giving means, variance and t values for mean effect (m), additive effect (d), dominance effect (h), additive × additive interactions (i), additive × dominance interactions (j) and dominance x dominance type interactions (l). The soft ware calculated the above parameters based on formulae:

(m) = mean genetic effect = that of F_2 generation for a morphometric measurement, (d) = Additive effect for a particular morphometric mean percentage value of all generations = the difference of B_1 and B_2 (B_1-B_2).

Table 1. Different scaled values of A. B, C and D with corresponding SE, variance and t values as per scaling scaling test of GMA.

Mean morphometric ratios in percentage of standard length	A ± SE: variance (t)	B ± SE: variance (t)	C ± SE: variance (t)	D ± SE: Variance (t)
Head length	-6.41± 2.71: 7.39 (-2.36)*	1.28 ±2.39: 5.73 (0.53)	3.15±3.33: 11.09 (0.94)	4.14±2.16: 4.67 (1.91)
Head depth	-4.98±1.98: 3.93 (-2.51)*	-3.76±1.23: 1.52 (-3.05)*	-6.24±4.33: 18.83 (-1.43)	1.25±2.31: 5.35 (0.54)
Head thickness	-1.24±1.83: 3.34 (-0.67)	-7.39±2.11: 4.46 (-3.49)*	-10.73±3.13: 9.85 (-3.41)*	-1.05±1.98: 3.92 (-0.53)
Body length	10.11±3.89: 15.19 (2.59)*	-1.23±2.13: 4.54 (-0.57)	-16.32±5.42: 29.42 (-3.01)*	-12.6±3.37: 1.39(3.73)*
Body depth	-5.74±4.38: 19.20 (-1.30)	-10.44±1.48: 2.2 (-7.03)*	-34.18±2.9: 8.40 (-11.78)*	-9.0±2.56: 6.569 (3.51)*
Body thickness	0.67±1.52: 2.31 (0.44)	-4.07±1.42: 2.03 (-2.85)*	-17.8±2.55: 6.52 (-6.97)*	-7.2±1.54: 2.38 (-4.66)*

**A, B, C and D: Different scales of genetic interactions, values in parentheses are the t values, * Significant at P, 0.05; A, $2B_1 – P_1-F_1$ and V_A, 4V (B_1) +V (P_1) +V (F_1) where $B_{1:}$ indicates the mean value of the character of the 1st backcross generation $P_{1:}$ presents the value of 1st parent (catla) $F_{1:}$ presents that of F_1 hybrids V (Xn): corresponding values of variances B= $2B_2-P_2-F_1$, V_B=4V (B_2) +V (P_2)+V (F_1); C=$4F_2-2F_1-P_1-P_2$, V_C = 16V (F_2)+4V(F_2)+V (P_1)+V (P_2) D = $2F_2-B_1-B_2$, V_D = 4V(F_2)+V (B_1)+V(B_2).

Table 2. Values of genetic interactions namely,- m, d, h, i, j and l ± standard deviation and type of epistasis.

Ratios	m	d	h	i	j	l	Type of Epistasis
Head length	37.96±4.37*	4.18±0.65*	-0.93±11.50	-8.28±4.32*	-3.84±1.76	13.41±7.0	Duplicate
Head depth	25.86±4.64*	4.26±0.39*	0.84±10.41	2.5±4.62	0.61±1.10	11.24±5.99	Complementary
Head thickness	16.57±3.89*	0.47±0.45	4.21±9.94	2.1±3.96	3.07±1.38*	6.53±6.11	Complementary
Body length	45.23±6.77*	-3.97±0.48*	58.28±16.60*	25.2±6.75*	5.67±2.19*	34.08±10.11*	Complementary
Body depth	14.36±5.15*	3.75±0.51*	20.91±14.38	18.0±5.12*	2.35±2.28	-1.82±9.37	Duplicate
Body thickness	3.43±3.10	-0.87±0.31*	25.14±7.60*	14.4±3.08*	2.37±1.02*	11.0±4.64*	Complementary

*** m: mean genic effect (F_2); V (m) = V $F_{2:}$ d: additive effect (B_1-B_2); V (d) = V B_1 + V $B_{2:}$ h: dominance effect (F_1- $4F_2$- ½ P_1 -½ P_2 + $2B_1$ +$2B_2$); V (h) = V F_1 + 16 V F_2 + ¼V P_1 + ¼V P_2 + 4VB_1 + 4V$B_{2:}$ i: additive x additive interactions ($2B_1$ +$2B_2$ -4 F_2); V (i) = 4V(B_1)+ ¼V(B_2)+ 16V(F_2); j: additive x dominance interactions (B_1- ½ P_1 -B_2 + ½ P_2); V(j) = V(B_1) +¼V(P_1)+ V(B_2)+ ¼V(P_2); l: dominance x dominance interactions (P_1+ P_2+ $2F_1$+4 F_2- $4B_1$-$4B_2$); V(l) =V(P_1)+ V(P_2)+ 4V(F_1)+16V(F_2) +16V(B_2)+ 16V(B_1); Significant at P = 0.05.

The value of h (dominance effect) was calculated by the mean percentage values of F_1-$4F_2$-1/2P_1-1/2P_2+$2B_1$+$2B_2$. Their corresponding variances were calculated as V (m) = V (F_2), V (d) = V (B_1) + V (B_2), and V (h) = V (F_1) + 16 V (F_2) + ¼ V (P_1) + ¼ V (P_2) + 4 V (B_1) +4 V (B_2). The values for all the three genetic interactions are: (i) = $2B_1$+$2B_2$ -4 F_2 and V (i) = 4 V (B_1) +¼ V (B_2) + 16 V (F_2). Similarly, (j) = B_1-1/2 (P_1)-B_2+ ½ (P_2) and V (j) = V (B_1) + ¼ V (P_1) + V (B_2) + ¼ V (P_2) and (l) = P_1 + P_2 + $2F_1$ + $4F_2$-$4B_1$-$4B_2$ and V (l) = V (P_1) + V (P_2) + 4 V (F_1) + 16 V (F_2) + 16 V (B_2) + 16 V (B_1).

Karyomorphology

After acclimatization, the individuals were subjected to starvation for 24 h in separate aquarium and were injected with Concanavalin-A (1mg/ml in Phosphate Buffer Saline PBS of pH 7.4 @ 1ml/100 g body weight prior to 48 h of dissection). Second dose was administered 24 h prior to actual sacrifice. Then they were injected with colchicine @ 1ml/100 g body weight 2 to 3 h prior to dissection. Kligermann and Blooms (1977) method was adopted followed by flame drying and stained in 4% Giemsa working solution (BDH). Counting of chromosome number and assignment of karyomorphologies to each set of chromosome was done following the study of Levan et al. (1964).

Esterase profiling

According to the study of Abersold et al. (1987), Esterase (EC: 3.1.1.) was profiled by vertical slab gel (5% native polyacrylamide gel electrophoresis) in discontinuous buffer system (Rechardson et al., 1986) using Bio-Rad made mini electrophoretic apparatus (Mini Protein-II, Catalogue no 165) at 4°C with running voltage of 250 V for 2 h. The co-dominant isozyme marker bands were assigned different codes for different loci like aa, AA, BB and bb for homozygous genotype and AB Ab, aB, bB, ab, Aa for heterozygous genotypes. The data based on isozyme polymorphism of esterase markers were analyzed and the genetic distance matrix was constructed by GENEPOP-3.2.

PCR-RAPD generated Molecular Marker analysis

Extraction of genomic DNA was done by Sambrook et al. (1989) with suitable modification (Barman et al., 2003), following the routine protocol of phenol-chloroform- isoamyl alcohol. Polymerase chain (PCR) reactions were set up in sterile environment by 5 pmol of selected primers (oligonucleotide decamers) and 0.5 U of Taq DNA to amplify in thermal cycler Gene Amp 9700 (Applied Biosystems). Initial denaturation at 94°C (4.0 min) was followed by 35 cycles in 1.0 min (94°C), 1.0 min (36°C) and 2.0 min (72°C). Final extension for 7.0 min at 72°C was allowed. The experiments were conducted for 10 sets decamer random primers. The amplified DNA was subjected to 1.8 % agarose gel electrophoresis along with 100 bp DNA ladder or gene ruler (FERMENTAS: MBI #SM0321) and were visualized under UV light using gel documentation system (Uvi tech, Techne, UK). Band scoring was done as 0 for absence or 1 for presence of bands by Quantity One (BIO-RAD) and was analyzed by computer-simulated software POPGENE 32 for construction of dendrogram based on genetic distance matrices according to the study of Nei (1972, 1978) by Unweighted Paired Group Method with Arithmetic Averages (UPGMA).

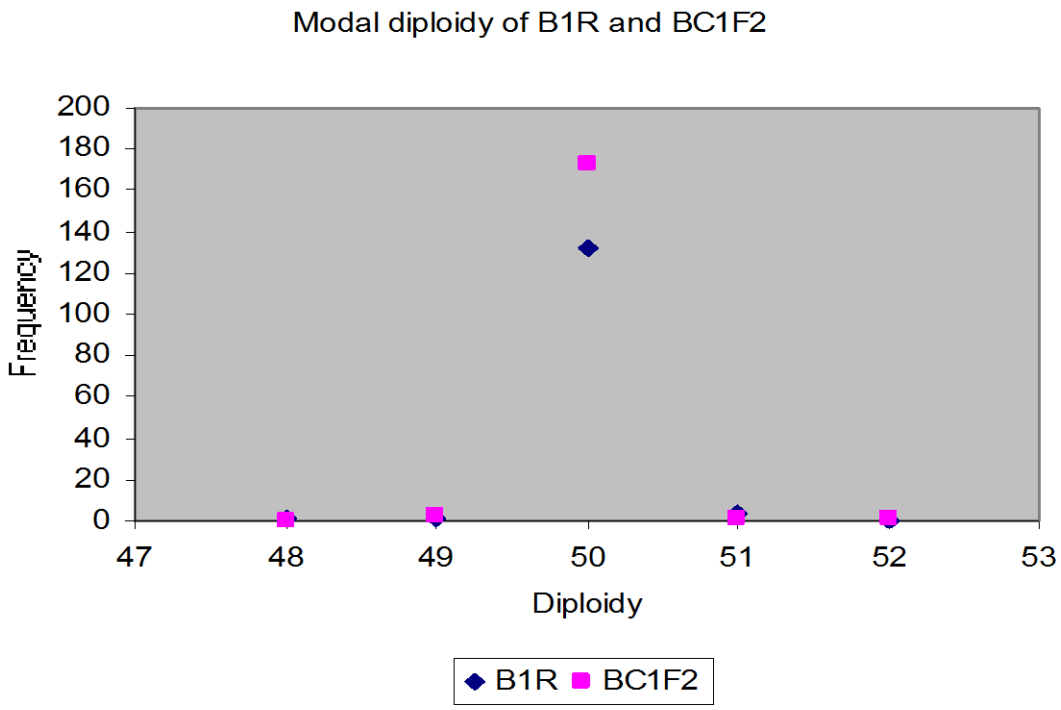

Figure 1. Modal diploidy (2n = 50) in backcrosses of catla and rohu.

Table3. Comparative karyomorphologies of backcrosses and parental carps.

Carp	Chromosomal morphologies						Reference
	2n	FN	M	Sm	St	T/A	
Catla	50	78	12	16	-	22	Zhang and Reddy (1991)
Rohu	50	-	6	16	8	20	Nagpure (1997)
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	*

******** FN, Fundamental number; M, metacentric; Sm, sub-metacentric; St, sub telocentric; T/A, telocentric/anacentric; ***,** As per the present findings; Sample size (n), B_1R (15) and BC_1F_2 (17).

The soft ware POPGENE (Yeh et al., 1999) calculated genetic distances and identities based on the formulae of pair-wise similarities (S_{AB}) as per Lynch (1990) originally based on Nei (1972) and Nei (1978) for similarities index, using the data from polymorphic primers where $S_{AB} = 2\ N_{AB}/(N_A+N_B)$, where N_{AB} is the number of DNA fragments between individuals A and B, and N_A and N_B are the total number of fragments possessed by individuals A and B. The mean pair-wise similarity S was computed as $S = \Sigma S_{AB}/n$ where it is the arithmetic mean of all S values. As per Lynch (1990), the variance of S was calculated as V (S) = 2S (1-S)(2-S)/ N(4-S). N stands for average number of DNA fragments per individual.

RESULTS

Morphometry and generation mean analysis

Six mean morphometric proportions namely:- length, depth and thickness of head and body were analyzed in backcrosses. The head morphometries were observed intermediate to parental catla and rohu but akin towards rohu, whereas body morphometries were more similar towards catla. The B_1 progenies showed four characters that is, length and thickness of head as well as length and depth of body more similar to parental rohu where as B_2 backcrosses showed four ratios namely, length and depth of head, as well as, length and thickness of body of rohu. The B_1R backcrosses showed head thickness like catla where as body depth like rohu and BC_1F_2 showed two characters like depth and thickness of head similar to rohu. The mean head length in percentage of standard length for parental rohu in current study was found similar to those in earlier observations of Chondar (1985), whereas it was highest for catla and lowest for rohu.

As per the scaling scaling test (Table 1) of generation mean analysis, the generation means for head length from

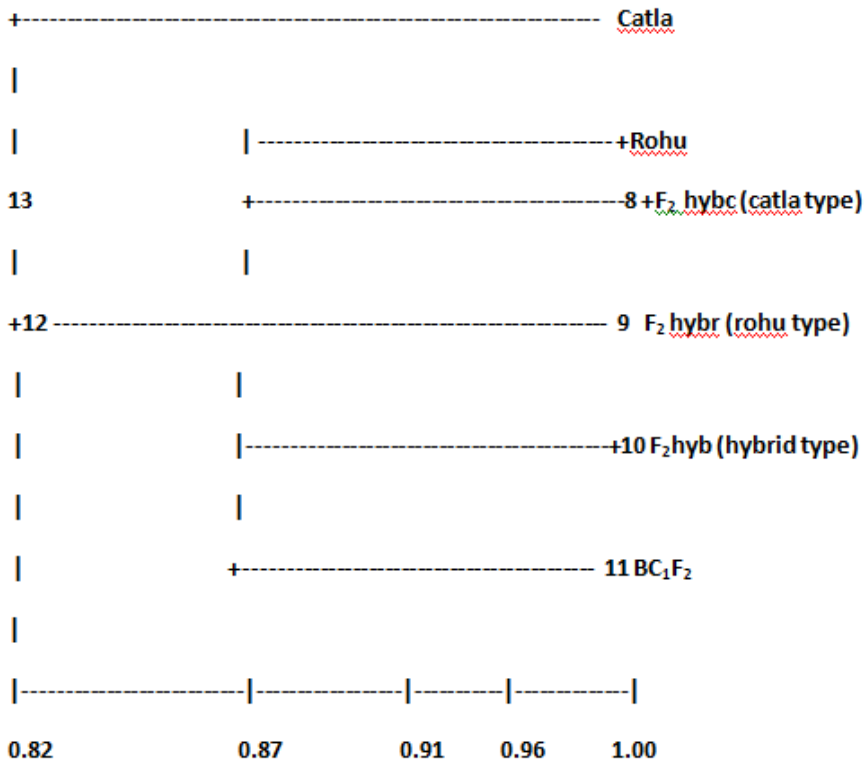

Figure 2: Dendrogram based on esterase profiling.

all generations showed the only significant value of scale A whereas generation mean morphometries for depth, scale A and B were found significant. Scales B and C differed significantly for thickness. Out of all the four scaled values for body length, only B was found non-significant and other three scaled values were significant. For body depth parameters in all generations, three scales namely, B, C and D differed significantly. Body thickness showed the only non-significant scaled value of A.

For the six parameter model presented in (Table 2), head length resulted in three significant parameters namely:- mean effect (m); additive effect (d) and additive x dominance epistasis (i) but that for the head depth, only m and d were significant. Similarly for head thickness, the significant parameters were mean genic effect (m) and additive x dominance interactions (j). All parameters were found significant for body length where as body depth showed significant parameters of m, d and i. All parameters for body thickness were significant except the mean genic effect (m).

Karyomorphologies

The backcross progenies in the present finding confirm same diploidy as earlier observation in catla, rohu and F_1 (Figure 1 and Table 3). The number of metacentric chromosomes is 14 in B_1R and 8 in BC_1F_2. Similarly, the number of sub-metacentric in B_1R is 10 and 12 in BC_1F_2. Number of sub-telocentric chromosomes in B_1R and BC_1F_2 are 10 and 22. The number of telocentric/acrocentric chromosomes is 8 in BC_1F_2 and 16 in B_1R.

Esterase profile

There exists positive correlation for genetic distance between both the backcross generations and B_1R backcrosses were more distantly correlated to rohu then BC_1F_2 (Figure 2). Highest value of genetic distance of rohu was observed with catla followed by BC_1F_2, F_2 and B_1R. B_1R backcrosses showed maximum distance with catla and minimum with F_2. The BC_1F_2 generation showed maximum genetic distance with catla followed by rohu, B_1R and F_2.

PCR-RAPD based molecular markers

A total of 105 (one hundred and five) bands in all generations were analyzed where an average of 6 to 8 bands per primer was amplified. Primers like OPY-7 and OPY-12 amplified highest number of DNA fragments. The size range of amplified DNA fragments was 3000 to 300 bp. Almost 35.23 % of the total amplified bands were either catla or rohu specific and only 40 to 100% of them were polymorphic. Catla was found having maximum genetic identity with B_1 and minimum with F_1. That in ascending order was with rohu, BC_1F_2, B_2, B_1R and F_2.

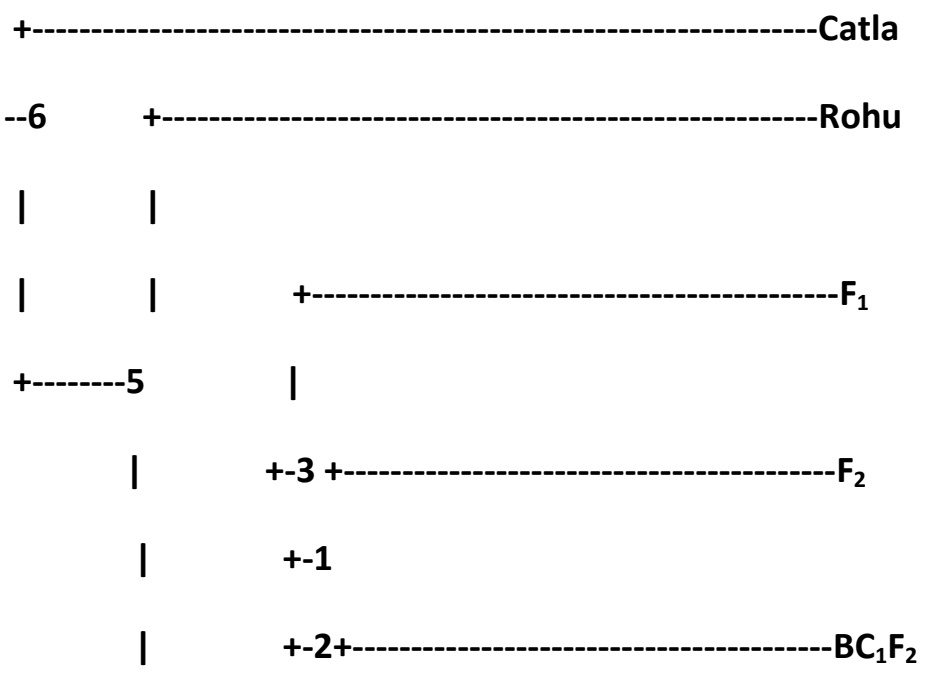

Figure 3. Dendrogram based on dominant PCR-RAPD markers.

Similarly, the genetic identity of rohu with carps in ascen-ding order was with catla, B_1, B_2, B_1R, F_2, BC_1F_2 and F_1 respectively and those of B_1R in ascending order was with catla, B_1, rohu, B_2 and BC_1F_2 respectively (Figure 3). Finally, the genetic identity of BC_1F_2 in ascending order was catla, B_1, rohu, F_2, F_1, B_2 and B_1R.

DISCUSSION

Selection in backcross programmes is used to either improve the genetic value of plant and animal populations or fine map quantitative trait loci. Both cases are helpful in understanding of the genetic bases of quantitative traits variation. Development of backcross progenies in Indian major carps that is, catlta, rohu and mrigal is not a well adopted practice as they have long generation cycle of three years approximately. But similar attempts were made in some other carps and non-carps earlier (Beherends et al., 1988; Jayaprakash et al., 1988; Andersons and Collins, 1995; Galbreath and Thorgaard, 1995) for various purposes of aquaculture in general; as well as, to understand their genetics. The present study was meant to gain inside to the carp genetics with respect to catla and rohu, as they contribute a lion share to the Indian market of freshwater captive fisheries. Backcrossing is a well-known and long established breeding scheme where a characteristic is introgressed from a donor parent into the genomic background of a recurrent parent (Hospital, 2005). Backcrossing is also useful to dissect the genetic architecture of quantitative traits because it isolates a gene, or chromosomal region, in a different genetic background (the genetic background of the recurrent parent). In fact, it is one of the few reliable methods to validate the additive effect of a quantitative trait locus (QTL) or a candidate gene. The experiments were conducted to introgress rohu characteristics with narrower head to hybrids and backcrosses.

Overall behavior of B_1 backcrosses in the present study was similar to those of catla being easy to be caught in net but those of other backcrosses were closer to rohu. The mean head length in percentage of standard length for parental rohu in current study was found similar to those in earlier observations of Chondar (1985), where as it was highest for catla and lowest for rohu. The values of head depth in percentage of standard length were observed highest for catla and lowest for rohu so also the same for body length in percentage of standard length Body depth in percentage of standard length in the present study were lower than those reported earlier (Chondar, 1985). As per Falconer and Mackay (1996), the scaling scaling test informs that significance of any of the four scales that is, A, B, C or D indicates the presence of epistasis and the type of epistasis is revealed by the significance of specific scaled values. Significance of A and B scales indicate presence of all the three types of genetic interactions such as:

i. additive × additive,
j. additive × dominance and
l. dominance × dominance interactions.

Similarly, significance of scaled value C suggested dominance × dominance interactions (j) and that of D scale value indicates additive × additive genetic interaction (i). Significance of both C and D scaled values indicate pre-

Table 4. Detail of induced breeding of various generations of carps.

Hormones				Egg release (Lt)	Weight(kg)	Spawn recovery(thousand)	Carps
♀	♂	♀	♂				
1	2	3	4	5	6	7	8
C	R	PGE	PGE	11.0	0.4	5.0	F_1
C	R	PGE	PGE	20.0	2.4	180.0	F_1
CR	CR	OVP	OVP	5.0	0.2	1.0	F_2
CR	C	PGE	PGE	4.5	0.3	5.0	B_1
CR	C	PGE	PGE	8.0	0.6	12.0	B_1
C	R	PGE	OVT	18.0	2.1	135.0	F_1
CR	CR	PGE	PGE	28.0	2.6	460.0	F_2
CR	R	PGE	OVT	7.0	0.35	6.0	B_2
CR	R	PGE	PGE	21.0	1.30	46.0	B_2
CR	C	PGE	PGE	12.0	0.95	33.0	B_1
CR	CR	PGE	OVP	24.0	2.2	*	F_2
CR	R	PGE	PGE	21.0	1.30	53.0	B_2
CRXC	R	OVP	PGE	7.0	1.5	50.0	B_1R
CRXC	CRXC	PGE	PGE	10.0	1.2	180.0	BC_1F_2
CRXC	CRXC	PGE	PGE	14.0	1.8	225.0	BC_1F_2
CRXC	R	PGE	PGE	5.0	0.2	13.0	B_1R
CRXC	R	OVP	PGE	6.0	1.0	40.0	B_1R
CRXC	CRXC	PGE	PGE	4.0	0.8	45.0	BC_1F_2

C, catla; R, Rohu; CR, hybrid of catla female and rohu male; CRxC, B_1 backcross generation developed by crossing CR hybrid female and catla male; F_2, developed by crossing CR hybrid *inter se* breeding; B_2, developed by CR hybrid female x rohu male; B_1R, developed by crossing B_1 backcross female and rohu male; BC_1F_2, developed by crossing B_1 backcross female and male (*inter se* breeding); PGE, Pituitary gland extract; OVP, ovaprime; OVT, ovatide.

sence of both (i) and (j) interactions. In the present study it was found that significance of all four scaled values indicated the presence of all the three types of genetic interactions (i, j and l) for overall expression of head length in percentage of standard length in all the studied generations of catla, rohu and backcrosses. Same was the result for head depth in percentage of standard length, indicating complicated polygenic interactions. Similar result for head thickness in percentage of standard length due to complicated polygenic interactions indicated to give more emphasis on dominance × dominance interactions (i) while considering for selective breeding. This required more attention towards out breeding to obtain the desired percentage of head thickness in various backcross generations of catla and rohu. While considering the body morphometries as per the scaling scaling test of GMA

Table 5. Measurements of morphometric parameters in mean percentage of standard length (mm) with values in parentheses indicating Standard Error of Mean (SEM).

Ratio	P_1	P_2	F_1	F_2	B_1	B_2	B_1R	BC_1F_2
				In % of standard length				
Head Length	35.42 (0.39)	26.0 (0.6)	32.5 (32.1)	32.116 (0.5)	28.103 (0.04)	27.916 (0.72)	31.084 (0.27)	30.859 (0.33)
Head Depth	26.29 (0.04)	19.8 (0.3)	25.9 (0.26)	21.633 (0.9)	22.62 (0.5)	20.533 (0.43)	23.252 (0.18)	21.65 (0.47)
Head Thickness	18.85 (0.25)	15.6 (0.6)	18.52 (0.2)	16.9 (0.044)	16.16 (0.3)	16.17 (0.492)	18.935 (0.24)	16.15 (0.316)
Body Length	65.33 (0.89)	73.9 (0.6)	66.55 (0.6)	67.2 (0.60)	72.43 (1.0)	72.096 (0.69)	70.18 (0.429)	70.147 (0.58)
Body Depth	33.68 (0.67)	26.9 (0.5)	29.72 (0.4)	24.766 (0.8)	28.70 (1.0)	25.73 (0.499)	26.083 (0.22)	25.652 (0.47)
Body Thickness	17.30 (0.30)	17.0 (0.3)	16.1 (0.25)	13.23 (0.30)	18.18 (0.4)	15.9 (0.37)	15.072 (0.24)	17.24 (0.465)
C.P. Length	16.968 (0.2)	16.5 (0.1)	16.55 (0.0)	18.633 (0.0)	17.48 (0.3)	15.48 (0.21)	15.8 (0.06)	20.40 (2.505)
C.P. Depth	9.614 (0.33)	12.4 (0.1)	13.05 (0.0)	11.46 (0.16)	11.9 (0.09)	11.83 (0.049)	11.77 (0.041)	13.01 (0.107)
C.P. Thickness	9.958 (0.19)	6.7 (0.05)	8.13 (0.02)	7.16 (0.096)	7.91 (0.06)	6.45 (0.061)	7.844 (0.047)	9.11 (0.221)
Snout Length	9.76 (0.061)	9.1 (0.06)	10.5 (0.04)	9.91 (0.13)	9.86 (0.06)	10.116 (0.11)	8.8 (0.016)	11.394 (0.08)
Eye Diameter	6.78 (0.091)	6.01 (0.2)	7.72 (0.09)	6.56 (0.068)	7.17 (0.14)	8.183 (0.19)	7.261 (0.013)	8.494 (0.054)
Pre-Dorsal	49.21 (0.16)	46.9 (0.4)	49.77 (0.1)	46.53 (0.55)	46.4 (0.43)	47.25 (0.66)	48.97 (0.109)	46.521 (0.19)
Pre-Pectoral	30.93 (0.74)	25.5 0.4)	29.3 (0.18)	28.03 (0.33)	27.9 (0.27)	28.2 (0.34)	28.25 (0.10)	28.868 (0.19)
Pre-Pelvic	54.968 (1.3)	51.8 (0.2)	53.59 (0.2)	45.7 (0.932)	51.76 (0.2)	52.4 (0.47)	52.85 (0.42)	47.62 (0.674)
Pre-Anal	76.09 (1.63)	76.63 (1.25)	77.70 (0.2)	71.83 (2.4)	71.68 (1.3)	77.466 (1.59)	77.038(0.29)	73.536 (0.26)

P_1, Catla; P_2, Rohu; CP, Caudal Peduncle.

indicated significant values of B,C and D scales only for head depth in percentage of standard length, indicating the presence of additive x additive (i) and dominance × dominance interactions only. For body length and thickness in percentage of standard length all types interactions (i, j and l) were indicated by the scaling scaling test.

In the six parameter test model of GMA, the values of h and i having similar signs (+ ve or – ve) indicate presence of complementary epistasis and those with opposite signs indicate duplicate epistasis. According to the study of Viana (2000), the coefficient of the component (i) is same as the additive component (d) and the coefficient of the

Table 6. Genomic contribution from parents to offspring in various carp generations.

Carp Generation	Maternal Parent	Designation of the carp	Catla Genome (%)	Rohu Genome (%)
Catla	Catla	P_1	100	0
Rohu	Rohu	P_2	0	100
F_1 Hybrid	Catla	CxR or CR	50	50
F_2 Hybrid	F_1	CR x CR	50	50
B_1	F_1	CR x C	66	33
B_2	F_1	CR x R	33	66
B_1R	B_1	CRC x R	50	50
BC_1F_2	B_1	CRC x CRC	66	33

component (j) is same as the dominant component (h) in any generation.

The probable reason for shifting of head morphometry towards rohu type producing backcrosses with rohu like head (narrower) can be attributed to a probability of masking genes for head morphometry in rohu genome though dosage wise higher from rohu parents only in parental rohu and B_2 backcross only (Table 6). This is true when such morphometric characters are not monogenic but polygenic trait with complicated genetic interactions. For head morphometry, involvement of more than one gene present in rohu genotype to make the head narrower and less deeper contributing more for the expression in subsequent generations of catla and rohu. But for body morphometry which is another polygenic trait, the rohu genome itself is not getting fully expressed and the catla genome masking partially.

It is also apparent in Table 6 that contribution of parental catla genome as a whole is more in case of parental catla (100%), B_1 (66%) and BC_1F_2 (66 %) but higher whole genome contribution with parental rohu alike in cases of rohu (100%) and B_2 (66%) which is not showing any clear trend to confirm more genetic proximity of rohu with other subsequent generations. But, considering the contribution of maternal genome with rohu linked genes, it is significant that rohu alike genome present to some degree in maternal parents of rohu and also in cases of production of F_2, B_1 and B_2 where F_1 is the maternal parent and F_1 itself is having genetic dose from rohu parents. Similarly, for the development of B_1R and BC_1F_2 generations, the maternal parents are B_1 backcrosses with rohu genome's contribution. This might have induced the rohu genome to mask the catla genome for development of head morphometry but not for body morphometry.

Hybrids between Atlantic salmon (*Salmo salar*, At) and brown trout (*Salmo trutta*, Bn) were highly viable and expected to be functionally sterile due to major interspecific karyotypic differences (Galbreath and Thorgaard, 1995). In contrast to this in the present study, intergeneric differences of catla (genus Catla) and rohu (genus Labeo) has not produced similar results but all the hybrids and backcross generations developed were highly viable and fertile due to chromosomal compatibility with same diploid number. The karyotype of B_1R and BC_1F_2 progenies in the present findings show 2n=50 with differences in their karyomorphologies. The rough estimation of metacentric and submetacentric chromosome (M + Sm) in catla is 28 and in rohu 22. Similarly, estimation of sub-telocentric and telocentric chromosome (St + T) which looks similar and difficult to identify is 22 in catla and 28 in rohu. Both comparative estimations are just opposite to each other in catla and rohu parents. Keeping these facts in view, the (M+Sm) value in all generations are 22, 24 and 20 in F_1, B_1R and BC_1F_2 generations alike rohu. So also the values of St + T are 28, 26 and 30 in F_1, B_1R and BC_1F_2 generations alike rohu. Although these are very crude estimations not to be ascertained with full conformity but points more towards genetic proximity with rohu parents. However, stronger and more authentic comparison application of chromosomal banding techniques could have served better. But as a first hand or preliminary information at chromosomal level of investigation through karyotyping it can be accepted that, the genomic organizations of different carp generations are in more proximity with rohu genome.

The dendrogram for esterase profile based on genetic distance matrix showed two distinct clusters of carps linked together where one with rohu and excluding catla keeping aside in a separate branch. From this observation it was clear that, rohu parents are more close to all the subsequent generations of carps rather than the catla parents. Hence, all generations of carps were found more linked to rohu than catla. In the phylogenetic tree based on PCR-RAPD markers, two distinct groups of carps were observed where rohu was found along with all carps except catla keeping aside in a separate branch, similar to earlier observations as per esterase marker. The overall genetic evaluation showed more proximity of backcrosses with rohu rather than with catla. A possible explanation ascribed is due to disruption of co-adapted gene complexes or alteration in dominance relationships between alleles. Such disruption might be brought by the differences for the alterations in various components of development of hybrids and backcrosses as compared to parental stock that is, catla and rohu. For head morphometries it is desirable and must be welcomed in future perspectives for freshwater aquaculture in Indian peninsula.

A correlation test among genetic distance matrices of molecular and biochemical marker showed a coefficient of 64%. The success of breeding the backcrosses and their genetic evaluation to find out the heredity may be attributed to nature itself, which accepted intra-generic hybridization resulting in viable hybrids due to their compatibilities at chromosomal level. The dominance of rohu head morphotypes over catla was considered to be of high significance from aquaculture point of view. The trend of morphometric evaluation is appropriately supported by findings from isozyme and molecular marker analysis. The type of inheritance exhibited by these backcrosses of catla and rohu has not been reported before but appears similar to that reported in backcrosses of other vertebrate species. These results show the importance of backcrossing as well as use of genetic markers to determine parental contributions and gene segregation in species hybrids.

REFERENCES

Abersold PB, Winans GA, Teel DJ, Milner GB, Utter FM (1987). Manual for starch gel electrophoresis: A method for the detection of genetic variation. NOAA Technical Report, N.M.F.S.61, December 1987, W.A. pp. 1-19.

Anderson DM, Collins JJ (1995). In: Proceedings of International Conference on Restoration of Lake Trout in the Laurentians great lake. J. Great Lakes Res**.** 21(1): 260-266.

Balon EK (1995). Origin and domestication of the wild common carp: from Roman gourmets to the swimming flower. Aquacult. 129(5): 3-48.

Barman HK, Barat A, Yadav BM, Banarjee S, Meher PK, Reddy PVGK, Jana RK (2003). Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. Aquacult. 217:115-123.

Basavaraju Y, Devraj KV, Ayyar SP (1995). Comparative growth of reciprocal carp hybrids between *Catla catla* and *Labeo fimbriatus*. Aquacult. 129:187-191.

Beherends LL, Kingsley JB, Price A H (1988). In: The Second International Symposium on Tilapia Aquaculture, Bangkok, Thailand, 16-20 March, 1987, 15: 125-133.

Chondar SL (1985). Systematic account of carp hybrid Nadina II *Labeo rohita* male X *Catla catla* female. J. Inland Fish. Soc. India 17 (1& 2): 66-70.

Falconer DS (1981). Introduction to Quantitative Genetics. 2nd Edition, Longmann, London and New York, pp: 338-342.

Falconer DS, Mackay TFC (1996). Introduction to Quantitative Genetics. DS Falconer and TFC Mackay (eds.) 4th edition, Longmann Publication, Addision Welseley Longmann, Ltd., Essex, England, pp 125.

Galbreath F, Thorgaard GH (1995). Sexual maturation and fertility of diploid and triploid Atlantic salmon X brown trout hybrids. Aquacult. 137 (1-4): 299-311

GENRES (1994). Data entry module for genres statistical software pascal intl software solution. Version 3.11.

Haymann BI, Mather K (1955). The description of genetic interactions in continuous variation. Biomet. 11: 69-82.

Hospital F (2005). Selection in backcross programmes**.** Phil. Trans. R. Soc. B: Biological sciences, 29 July 2005, 360 (1459): 1503-1511.

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

Jana RK (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 pp. 50-60

Jayaprakash V, Tave D, Smitherman RO (1988). In: The Second International Symposium on Tilapia Aquaculture, Bangkok, Thailand, 16-20 March, 1987, 15:197-201.

Jhingran VG, Pullin, RSV (1985). A hatchery manual for the common, Chinese and Indian major carps. ICLARM studies and Reviews 11, pp.191-192.

Kligermann AD, Bloom SE (1977). Rapid chromosome preparation from solid tissues of fishes. J. Fish. Res. Board Can. 34: 266-269.

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

Lynch M (1990). The similarity index of DNA finger printing. Mol. Biol. Evol. 7: 478-484.

Mather K, Jinks JL (1982). Genetical foundation. In: Biometrical genetics, Third Edition, Chapmann and Hall, London, pp: 32-33.

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

Nei M (1972). Genetic distances between populations. The Am. Nat. 106: 283-292.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89: 583-590.

Phanse VG, Sukhatme PV, 1995. Statistical methods for agricultural workers. 4th Edition, Revised by PV Sukhatme and VN Amble, Published by Dr. R.D. Sharma, Elegant Printers, Mayapuri, New Delhi, 48-95.

Rechardson BJ, Baverstock PR, Adams M (1986). Allozyme electrophoresis, a hand book for animal systematic and populations studies, Academic Press, pp. 410-411.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: a lab manual series. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York. Vol I, II and III

Tripathy SK, Gaur KK, Sarangi N (2010). Generation mean analysis for head and body morphometries of *Catla catla* (Ham.) and *Labeo rohita* (Ham) backcross progenies (B_1 and B_2). Natl J. Life Sci. 7(2):137-140.

Viana JMS (2000). Generation Mean Analysis in relation to polygenic systems with epistasis and fixed genes. Pesq. Agropec.Bras, Brasilia, June 2000, 35(6): 1159-1167.

Yeh FC, Yang RC, Boyle T (1999). POPGENE version 1.31: (http://www.ualberta.ca/~fyeh).

Zhang SM, Reddy PVGK (1991). On the comparative karyomorphology of three Indian Major Carps *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.). Aquacult, 97: 7-12.