

Comparative Genetic Diversity in Natural and Hatchery Populations of Indian Major Carps (*C. catla* and *L. rohita*)

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ABSTRACT : This study deals with the characterization of three populations (two hatchery and one natural) of Indian major carps *Catla catla* and *Labeo rohita* from different locations in India. The genetics of Indian major carps has been completely obscure and this is the first report on comparative allozyme variations in natural and hatchery population. The total 10 biochemical genetic markers used to measure interspecific and intraspecific level of diversity. The allele frequency data indicate different level of genetic variability in three populations. The hatchery population exhibited least polymorphism, low level of heterozygosity and genetic diversity. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 9 : 1197-1203)

Key Words : Variation, Allozyme, Diversity, Polymorphism, Cultured, Wild, Indian Major Carps

INTRODUCTION

The Indian major carps catla (*Catla catla*, Hamilton) and rohu (*Labeo rohita*, Hamilton) being an excellent experimental model as they grow faster than other indigenous carps. Together these carps account for approximately 75% of the total inland aquaculture production in India (Barman et al., 2003). For many centuries major carps have been cultured in several northeastern states of India and play an important role in the fisheries and aquaculture of tropical and sub tropical regions of India.

Taxonomically, carps belong to the family Cyprinidae (Berg, 1940). The major carps have been extensively studied for many years, however, comparatively little efforts have been made to study its genetic aspect. Although a good number of studies have been conducted on the genetic characterization of fish abroad (Hunter and marker, 1957; Lewontin and hubby, 1966; Allendorf and utter, 1979; Smith and conroy, 1992). Little information is presently available on fishes in India (Menezes, 1993; Padhi, 1994).

Genetic markers come in a variety of formats in modern molecular biology, although initial marker systems were based on protein polymorphism and morphological characteristics. There is a wide array of DNA based molecular marker types (Davis and Hetzel, 2000). Isozyme electrophoresis, restriction fragment length polymorphism (RFLP) and microsatellites have been so far used to analyze genetic similarity and diversity in genetics and breeding research of fish/invertebrates. Also, molecular markers from random amplified polymorphic DNA (RAPD) have recently

been used to evaluate genetic diversity and/or similarity in several organisms (Yoon and Park, 2002).

The advent of gel electrophoresis technique of allozyme protein brought the electrophoresis separation of proteins together with the specificity of histochemical detection of protein product of single loci. Electrophoretic technique in biochemical genetic has contributed significantly in understanding the genetic variation existing in natural as well as hatchery population of *C. catla* and *L. rohita*.

In the following presentation, the use of isozyme electrophoresis for the assessment of genetic diversity was carried out. The major carp was chosen for the study because more is known about genetic control of the growth of carp than of any other fish species. The cultivated major carp has been selected by Asian fisheries breeders for hundreds of years and is considered a truly domestic breed.

In the present study, the number of fishes representing *C. catla* and *L. rohita* ranged from 30-50 and 40-60 respectively to compare their genetic diversity in natural and hatchery populations. There are no published reports on the biochemical genetic characterization of natural and hatchery populations of *C. catla* and *L. rohita* population existing in India.

MATERIALS AND METHODS

Animals

Three populations of *C. catla* and *L. rohita* were live collected from different Indian localities. The hatchery populations were of fresh water fish farm Powerkheda (M.P.) and Bhatkal fish farm (Haryana) and the wild population was of Yamuna river, Delhi. Figure 1 shows the different locations of these areas. The live samples, weighing 100-150 gms were collected and maintained in separate plastic pools equipped with aeration system. The sample size used from each locality is indicated in Table 1.

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Table 1. Sample size used in the study

Enzymes	CPK ^a	CBK ^b	CYM ^c	Total	RPK ^d	RBK ^e	RYM ^f	Total
MDH	20	17	7	44	15	19	20	54
AAT	20	13	7	40	15	19	20	54
EST	20	16	7	43	15	19	20	54
ADH	20	10	6	36	15	19	17	51
IDH	20	13	7	40	15	13	13	41
G6PD	18	13	6	37	15	12	20	47
LDH	18	15	7	40	15	20	17	52
PER	20	13	6	39	15	20	20	55
SOD	5	6	6	17	6	6	6	18

^a Powekheda population of *C. catla*; ^b Bhatkal population of *C. catla*; ^c Yamuna river (Delhi) population of *C. catla*; ^d Powekheda population of *L. rohita*; ^e Bhatkal population of *L. rohita*; ^f Yamun river (Delhi) population of *L. rohita*.

MDH: Malate dehydrogenase; AAT: Asparate amino transferase; EST: Esterase; ADH: Alcohol dehydrogenase; IDH: Isocitrate dehydrogenase; G6PD: Glucose 6 phosphate dehydrogenase; LDH: Lactate dehydrogenase; PER: Peroxidase; SOD: Superoxide dismutase.

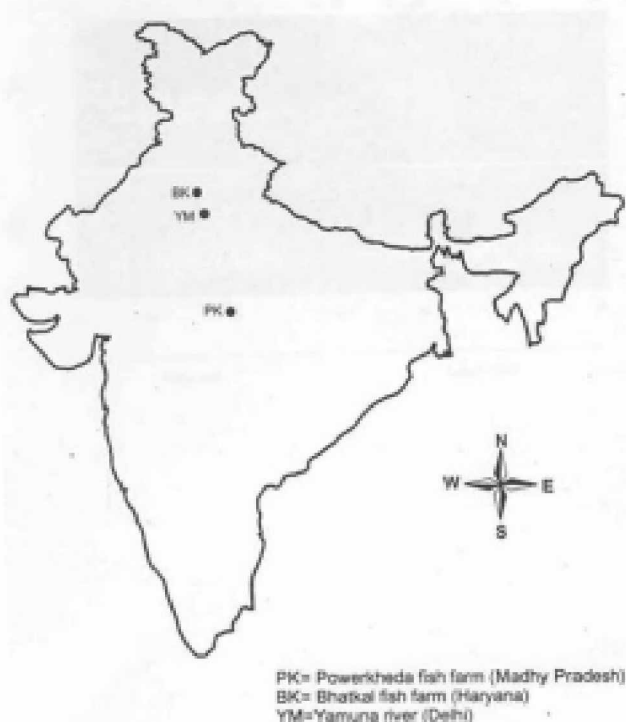


Figure 1. Map showing the locations of the three populations selected for the study.

Biochemical analysis

Heart, brain, eyes, liver and muscle tissues were dissected from live specimens. The homogenate prepared were kept at -70°C and used for biochemical analysis. A total of 15 variable biochemical genetic loci were analyzed. (Mdh-1, Mdh-2, Aat-1, Est-1, Est-2, Adh-1, Adh-2, Idh-1, Idh-2, G6PD-1, Ldh-1, Per-1, Per-2, Sod-1 and Sod-2).

Starch gel electrophoresis was performed on acrylic plate model with each marker having its own unique buffer system. Alleles were scored on the basis of their electrophoretic mobility from the most anodal to most cathodal bands.

Data analysis

Intra-specific diversity : The following estimates of genetic variation were calculated.

(i) Allele frequencies at the polymorphic loci: The allelic frequency data were used as input for further calculations. (ii) Number of alleles per locus (A_p): (number of loci polymorphic in at least one population / number of loci analyzed) (iii) Effective number of alleles (A_{ep}) (iv) Genetic diversity index (for each locus), H_{ep} : $1-(p_i^2)$. (v) Mean genetic diversity: Average of H_{ep} values over all loci (vi) Heterozygosity (H) unbiased for sample size.

Using the gene frequency data, Nei's genetic distance matrix was constructed. The PHYLIP package (VERSION 3.5c, Felsenstein, 1993) was used for various phylogenetic analysis. Nei's genetic distance was computed for all data sets using GENDIST program. All were further used to construct phylogenies using UPGMA and Neighbor Joining by NEIGHBOR program.

Inter-specific diversity : The following estimates of genetic variation were calculated.

(i) Mean number of alleles per locus (A_s). (ii) Percentage of polymorphic loci (P_s). (iii) Effective number of alleles (A_{es}). (iv) Gene diversity in the total population (H_t) was calculated. (v) Average genetic diversity of the population was calculated (H_s). (vi) Genetic diversity due to variations among population (D_{st}) was calculated $H_t - H_s$. (vii) To calculate the extent of differentiation among population, the coefficient employed was G_{st} (Nei, 1975) defined as D_{st}/H_t .

RESULTS

Intra-specific diversity

Protein polymorphism : Of the 15 genetic loci, 13 were found to be polymorphic showing electrophoretic variations in three wild and hatchery populations of *C. catla* and *L. rohita*. These loci were Mdh-1, Mdh-2, Aat-1, Est-1, Est-2, Adh-1, Adh-2, Idh-1, G6pd-1, Ldh-1, Per-1, Per-2 and Sod-1. Three loci Ldh-1, Per-1 and Per-2 were highly variable

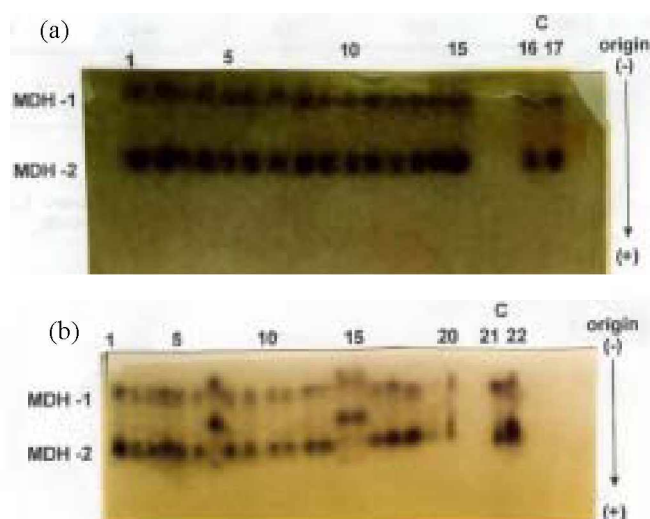


Figure 2. Represents MDH profiles resolved with 0.072 M. Histidine citrate, pH 6.5 buffer using eye tissue of *C. catla* and *L. rohita* in different populations. (a) shows MDH profile of fifteen individual fish samples of *L. rohita* from Powerkheda population. Lanes 16 and 17 indicated profiles in samples of *C. catla* used as internal standard. (b) shows MDH profile of twenty individual fish samples of *C. Catla* from Bhatkal population. Lanes 21 and 22 indicated profiles in samples of *C. catla* used as internal standard. The alleles have been indicated with → sign.

showing the presence of 5 alleles in Ldh-1 and 3 alleles in Per-1 and Per-2. Loci that were found to be moderately polymorphic were Mdh-1, Mdh-2 (Figure 2 a,b). Aat-1, Est-1, Est-2, Adh-1, Adh-2, Idh-1, G6pd-1 and Sod-1 segregated for two alleles.

The allele frequencies observed at 15 loci in *C. Catla* and *L. rohita* populations are given in Table 2. The *C. catla* and *L. rohita* populations shows a high frequencies for the Ldh1/2 (0.83), Per1/1 (0.75) and Per-2/2 (0.75) in hatchery populations of Bhatkal and Powerkheda and wild population from Yamuna river (Delhi) respectively in *C. catla* and Adh-2/1 (0.98) Est1/1 (0.94), Aat1/1 (0.94) and Adh1/1 (0.80) in the Powerkheda population in *L. rohita* population. No variation was seen at two biochemical markers, which were monomorphic. Sod-2 and Idh-2 in *C. catla* and at three loci Est-2, Idh-2 and Sod-2 in *L. rohita*.

Estimation of genetic variation

From Table 3 it is observed that the total amount of polymorphism is not distributed equally between hatchery

Table 2. Allele frequencies of three populations at 15 biochemical genetic loci

Locus	Alleles	CPK	CBK	CYM	RPK	RBK	RYM
Mdh-1	Mdh1:1	1.00	0.76	1.00	1.00	0.89	0.85
	Mdh1:2	0.00	0.24	0.00	0.00	0.11	0.15
Mdh-2	Mdh2:1	1.00	0.76	1.00	1.00	0.89	0.80
	Mdh2:2	0.00	0.24	0.00	0.00	0.11	0.20
Aat-1	Aat-1:1	0.00	0.77	0.00	0.06	0.16	0.95
	Aat1:2	1.00	0.23	1.00	0.94	0.84	0.05
Est-1	Est1:1	1.00	1.00	0.00	0.94	0.50	0.90
	Est1:2	0.00	0.00	1.00	0.06	0.50	0.10
Est-2	Est2:1	1.00	1.00	1.00	1.00	0.87	1.00
	Est2:2	0.00	0.00	0.00	0.00	0.13	0.00
Adh-1	Adh1:1	0.00	0.80	0.00	0.80	0.11	0.12
	Adh1:2	1.00	0.20	1.00	0.20	0.89	0.88
Adh-2	Adh2:1	1.00	0.00	1.00	1.00	0.97	0.97
	Adh2:2	0.00	0.00	0.00	0.00	0.03	0.03
	Adh2:3	0.00	1.00	0.00	0.00	0.00	0.00
ldh-1	ldh1:1	1.00	1.00	0.00	1.00	1.00	0.24
	ldh1:2	0.00	0.00	1.00	0.00	0.00	0.76
ldh-2	ldh2:1	1.00	1.00	1.00	1.00	1.00	1.00
	ldh2:2	0.00	0.00	0.00	0.00	0.00	0.00
G6pd-1	G6pd1:1	1.00	1.00	0.00	1.00	0.80	1.00
	G6pd1:2	0.00	0.00	1.00	0.00	0.20	0.00
Ldh-1	Ldh1:1	0.00	0.00	0.00	0.00	0.05	0.06
	Ldh1:2	1.00	0.83	1.00	1.00	0.45	0.44
	Ldh1:3	0.00	0.00	0.00	0.00	0.05	0.06
	Ldh1:4	0.00	0.17	0.00	0.00	0.45	0.44
Per-1	Per1:1	0.75	0.69	0.11	0.33	0.07	0.86
	Per1:2	0.25	0.31	0.67	0.67	0.50	0.14
	Per1:3	0.00	0.00	0.22	0.00	0.43	0.00
Per-2	Per2:1	0.44	0.34	0.10	0.16	0.50	0.00
	Per2:2	0.56	0.20	0.75	0.84	0.28	0.00
	Per2:3	0.00	0.46	0.15	0.00	0.22	1.00
Sod-1	Sod1:1	1.00	1.00	1.00	1.00	0.34	0.00
	Sod1:2	0.00	0.00	1.00	0.00	0.66	1.00
Sod-2	Sod2:1	1.00	1.00	1.00	1.00	1.00	1.00

and wild population. The intra-specific levels of individual variation (calculated over all 15 loci) are variables (Ap 1.13-1.53, Pp 0.13-0.46, Aep 1.00-1.33, Hep 0.05-0.18) for *C. catla* and (Ap 1.33-2.06, Pp 0.33-0.80, Aep 1.21-1.52 and Hep 1.12-1.52) for *L. rohita* species.

Bhatkal population of *C. catla* and *L. rohita* shows a high variability, suggesting the fact that a great deal of diversity exist here. However, the Powerkheda population shows a low variability. The heterozygosity values over 15 polymorphic loci (Table 2) indicated that among three populations, Bhatkal population showed higher heterozygosity values in both the species (*C. catla* 0.166, *L.*

Table 3. Estimation of genetic variation

Locality	Ap ^a		Pp ^b		Aep ^c		H ^d		Hep ^e		Av. H ^f	
	C	R	C	R	C	R	C	R	C	R	C	R
Pkheda	1.13	1.33	0.13	0.33	1.00	1.21	0.035	0.083	0.05	1.12	0.002-0.002	
Bhatkal	1.53	2.06	0.46	0.80	1.33	1.52	0.166-0.277		0.18	1.52	0.011-0.018	
Yamuna	1.26	1.93	0.13	0.73	1.10	1.34	0.126-0.223		0.05	1.34	0.008-0.014	

^a Mean number of alleles/ total number of loci; ^b Mean percentage of polymorphic loci; ^c Mean effective number of alleles; ^d Heterozygosity;

^e Mean genetic diversity at population level; ^f Average heterozygosity.

Table 4. Nei's genetic distance matrix of *C. catla* and *L. rohita* populations from various localities based on 15 biochemical genetic markers

	CPK ^a	CBK ^b	CYM ^c	RPK ^d	RBK ^e	RYM ^f
CPK	0.000					
CBK	0.218	0.000				
CYM	0.446	0.868	0.000			
RPK	0.067	0.175	0.477	0.000		
RBK	0.115	0.300	0.312	0.151	0.000	
RYM	0.168	0.223	0.527	0.259	0.230	0.000

^aPowerkheda population of *C. catla*; ^bBhatkal population of *C. catla*; ^cYamuna river (Delhi) population of *C. catla*; ^dPowerkheda population of *L. rohita*; ^eBhatkal population of *L. rohita*; ^fYamuna river (Delhi) population of *L. rohita*.

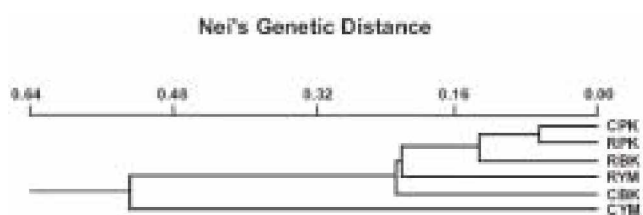


Figure 3. Dendrogram based on UPGMA analysis of isozyme data from three populations of *C. catla* and *L. rohita*.

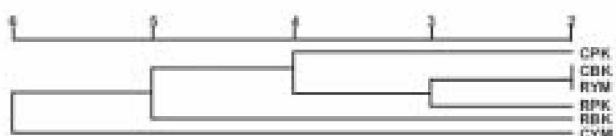


Figure 4. Neighbour Joining Tree based on Nei's Genetic Distance calculated from allozyme diversity.

L. rohita 0.277) than Powerkheda population (*C. catla* 0.035, *L. rohita* 0.083). This could be explained as Powerkheda being an isolated small breeding populations and the inbreeding could account for the lower level of heterozygosity. The average heterozygosity values also showed similar pattern in three populations.

Biochemical profile of *C. catla* and *L. rohita*

There are no published reports on the biochemical genetic profile of *C. catla* and *L. rohita* species. Using starch gel electrophoresis system, these species shows a distinct electrophoretic pattern. This means a relatively stable genetic make-up. Of the 15 biochemical genetic loci studied, 13 (Mdh-1, Mdh-2, Aat-1, Est-1, Est-2, Adh-1, Adh-2, Idh-1, G6pd-1 and Sod-1) were polymorphic. Three loci Ldh-1, Per-1 and Per-2 were segregate for four alleles and three alleles respectively and hence highly polymorphic. Ten loci (Mdh-1, Mdh-2, Aat-1, Est-1, Est-2, Adh-1, Adh-2, Idh-1, G6pd-1 and Sod-1) were moderately polymorphic segregated for two alleles. No gene variation was observed for the other two, which were monomorphic. Their allelic frequencies (data not shown) were used for construction of Nei's genetic distance matrix.

Table 5. Genetic diversity statistics (unbiased for sample size)

Locus	Hs ^a		Dst ^b		Gst ^c	
	C ^d	R ^e	C	R	C	R
Mdh-1	0.12	0.15	0.02	0.00	0.17	0.05
Mdh-2	0.12	0.17	0.02	0.01	0.17	0.07
Aat-1	0.11	0.15	0.26	0.31	0.69	0.66
Est-1	0.00	0.26	0.44	0.07	1.00	0.23
Est-2	0.00	0.07	0.00	0.00	1.00	0.09
Adh-1	0.10	0.28	0.28	0.19	0.72	0.40
Adh-2	0.00	0.03	0.44	0.00	1.00	0.01
Idh-1	0.00	0.12	0.44	0.25	1.00	0.67
Idh-2	0.00	0.00	0.00	0.00	1.00	0.00
G6pd-1	0.00	0.16	0.44	0.01	1.00	0.07
Ldh-1	0.09	0.39	0.01	0.17	0.17	0.29
Sod-1	0.00	0.14	0.44	0.19	1.00	0.56
Sod-2	0.00	0.00	0.00	0.00	1.00	0.00

^aMean genetic diversity within populations; ^bGenetic diversity due to variation among populations; ^cProportion of diversity among populations.

^d*Catla catla*; ^e*Labeo rohita*.

Genetic distance and cluster analysis

A matrix of genetic distance (Nei, 1978) was constructed from the allelic frequencies (Table 4). Nei's genetic distances range from a minimum of 0.067 (between Powerkheda population of *C. catla* and *L. rohita*) to a maximum of 0.886 (between Yamuna river, Delhi and Bhatkal population of *C. catla*). Genetic distances could reflect isolation by distance or some complex pattern of gene flow. The average genetic distance between *C. catla* and *L. rohita* was 0.263. Variation among populations of *C. catla* and *L. rohita* was measured by the average component, which was calculated as 0.254 and 0.106 respectively.

Dendrogram based on UPGMA analysis of allozyme data from three populations belonging to *C. catla* and *L. rohita* indicated close genetic similarity among the individuals of the two species (Figure 3). The respective populations of *C. catla* and *L. rohita* are well separated and except in case of *C. catla* of Powerkheda population which may be due to intermingling among the individuals of the two species in the Powerkheda hatchery.

A Neighbor joining tree based on Nei's genetic distance calculated from allozyme diversity (Figure 4) indicated the presence of greater genetic variation among the populations of *C. catla* and *L. rohita*. The *C. catla* population from Yamuna river, Delhi, which is wild population, is highly diverse and is not included in any cluster. The Powerkheda population of *C. catla* placed in the same cluster consisting of *L. rohita* population as in the UPGMA tree.

Inter-specific diversity

In the present study, the coefficient employed to measure the extent of differentiation is Gst. Table 5 shows the genetic diversity statistics calculated at 15 polymorphic loci. Dst (genetic diversity due to variation among population) is unbiased. The estimates of the Hs, in which

Table 6. Levels of allozyme variation

Level	P ^a		A ^b		H ^c		Level	Hs ^d		Gst ^e		Dst ^f	
	C	R	C	R	C	R		C ^g	R ^h	C	R	C	R
Population	0.24	0.62	1.31	1.77	0.10	1.32	Among populations	0.10	0.18	0.60	0.28	0.20	0.10
Species	0.46	0.86	1.60	1.13	-	-							

^a Percentage of polymorphic loci; ^b Mean number of alleles per locus; ^c Mean genetic diversity; ^d Genetic diversity within populations; ^e Proportion of total diversity among populations; ^f Genetic diversity due to variation among species population; ^g *Catla catla*; ^h *Labeo rohita*

certain highly variable loci such as Per-1 and Per-2 are mainly responsible for the increase in diversity in the entire population of both the species.

Table 6 gives a comparison of the species level and the population level variation. Mean genetic diversity at population level is nearly 10% in *C. catla* and 1.33% at *L. rohita* level with 24 and 62% loci polymorphic. The species is polymorphic at 46 and 86% isozyme loci examined. Genetic diversity due to variation among population (Dst) was related to the total diversity (Ht) to determine the proportion of diversity residing among populations (Gst). The Indian major carps *C. catla* and *L. rohita* populations shows a Gst values 0.60 and 0.20 respectively indicating high level of inter population heterozygosity in *C. catla*.

DISCUSSION

The Indian major carps generate interest in terms of its heterogeneity and population structure. Here a survey of electrophoretically demonstrable genetic variation in wild and hatchery populations (3 populations) was carried out. These populations were collected from different locations in India (Figure 1). A range of 30-50 and 40-60 fishes of *C. catla* and *L. rohita* respectively shows 33 alleles at 15 loci. These populations do not show any sub-species specific diagnostic biochemical profile but represent a complex admixture of different alleles and it is thus not possible to assign them to any one subspecies. The present study determines the extent of genetic variation within and among populations of *C. catla* and *L. rohita*. It was observed from the allele frequencies which shows the existence of unusually high level of polymorphism in *C. catla* (48%) and *L. rohita* (80%) population of Bhatkal fish farm. The Powerkheda (hatchery) population of both the species exhibited 1/3 of polymorphism being a genetically isolated population. Reduced isozyme polymorphism has been reported in some cultured stock of different fish species (Allendorf, 1975; Ferguson et al., 1985).

The two rare alleles were observed in wild population of Yamuna river (Delhi) and Bhatkal population of *L. rohita* from 33 alleles. The occurrence of rare alleles in both could be due to their common origin. It appeared that there has been some loss of rare alleles in hatchery population. Such losses may limit the adaptive potential of populations (Allendorf, 1986; Lande and Barrowlough, 1988). Changes

in allozyme gene frequencies, loss of rare alleles and lower than expected effective breeding numbers have been reported for hatchery stocks of abalone (Elliott, N. G. 2000). The occurrence of rare alleles was not seen in any population of *C. catla*.

There were differences in genetic diversity within and among populations. Bhatkal population of *L. rohita* exhibited higher genetic diversity and Powerkheda population of *C. catla* showed the lowest value. High variability may reflect intermixing with introduced form in Bhatkal fish farm. It may be desirable to protect a genetically divergent stock regardless of its level of variability. Nevo, 1978 stated that for evolutionary reason most organisms maintain high level of genetic variation. Ryman and Stahl, 1980; Ryman, 1981; Ryman and Stahl, 1981; Cross and King, 1983; Taniguchi et al., 1983; Vuorinen, J 1984; Allendorf and Phelps, 1988 reported genetic changes and loss of genetic variability in hatchery reared stocks of many fish species. The low genetic diversity in hatchery population of Powerkheda seems to indicate that the artificial production of fish might lead to a loss of genetic variation at allozyme loci within cultured population.

The Yamuna river (Delhi), a wild population showed high diversity being a large river offering a wide gene pool. The studies of Salmonids (Chicote et al., 1986) and brown trout (Vuorinen, 1984; Johansen et al., 1990; Fleming and Grass, 1994) also reported the similar behavior of wild and hatchery populations.

Development of domesticated and genetically improved strains is made possible through hatchery production. Propagation of genetically closed populations may, however, lead to loss of genetic diversity and inbreeding, with negative production effect (Elliott, 2000). Inbreeding is a cumulative phenomenon. Eknath, 1991 indicated poor growth performances of hatchery seed of carps in India. The result of our study confirms the previous finding by Maheswari and Birader, 1997 regarding inbreeding in *L. rohita* population of Powerkheda fish farm (hatchery). The two measures of heterozygosity are highly correlated, but this study focused on average heterozygosity (Kim et al., 2002). Low heterozygosity may be expected in case of isolated population of Powerkheda with low initial number and consequent genetic drift and inbreeding. High heterozygosity in Bhatkal may be due to mixing of fishes

during reproductive season. Bank et al. (1992) and Vuorinen (1984) compared hatchery and wild populations of *Clarias gariepinus* and trout respectively.

The calculation of a genetic distance between two populations gives a relative estimate of the time that has passed since the populations existed as single cohesive units. Small estimations of distance may indicate population substructure (i.e., subpopulations in which there is random mating but there is a reduced amount of gene flow). However, small estimation of distance may also be present because the populations are completely isolated but have only been separated for a short period of time. (Pandey et al., 2002) The minimum genetic distance between the species indicated that hatchery population of Powerkheda was a reproductive isolated population. Dilution of native gene pool through introduction of mixed origin hatchery has been reported by Belunke (1992) Ferguson and Mason (1981) in trout.

Genetic erosion and extinction threaten an increasing number of plant and animal species, the major consequence being a loss of global genetic diversity requires sets of genetic markers that characterize distinct populations (Yeo et al., 2002). The factors determining the genetic variability within and between have been of great importance to biologist (Lewontin, 1974). Diversity at species level is much more than at the population level indicating that both are rapidly evolving species. Much of the variation in genetic diversity among species is the result of both differences in the proportion of polymorphic loci and also observed that species level variation is not closely associated with the distribution of variation among populations. Comparison between genetic diversity of both the species revealed that *L. rohita* was more diversified genetically than *C. catla*.

Thus the Indian major carps *C. catla* and *L. rohita* present a classified case of a species in a highly active state of differentiation and evolution. Our study further showed the comparison of genetic variation between hatchery and wild population.

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