High level of hybridisation in three species of Indian major carps

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Abstract

Thirty individuals of each species of Indian major carps, i.e., *Catla catla*, *Cirrhinus cirrhosus* (*C. mrigala*) and *Labeo rohita*, obtained from a nursery near Mymensingh, Bangladesh were analysed by means of allozyme electrophoresis. Twenty-one loci were studied. Several loci revealed significant deviation from Hardy-Weinberg expectations caused by deficiency of heterozygotes, indicating Wahlund effects due to problems with species identification. Moreover, bimodal distributions of individual heterozygosity within the three putative species indicated hybridisation. This was confirmed using analysis of individual admixture proportions, as individuals misidentified to species and hybrids between species were observed. Furthermore, factorial correspondence analysis to visualize genetic relationships among individuals revealed three distinct groups containing misclassified individuals, along with some intermediate individuals interpreted as hybrids. Ten per cent of all *C. catla* and *L. rohita* had been erroneously identified to species, and 40 per cent of all presumptive *C. catla* were hybrids between *C. catla* x *C. cirrhosus* and *C. catla* x *L. rohita*. In the case of *C. cirrhosus*, 37 per cent of the samples were *C. cirrhosus* x *L. rohita* hybrids. Thirty per cent of all presumptive *L. rohita* turned out to be hybrids between *L. rohita* x *C. catla* and *L. rohita* x *C. cirrhosus*. The high incidence of hybrids in *C. catla* might be responsible for slower growth of the fish in aquaculture.

Introduction

Three years ago, the Danish aid-organisation (DANIDA) gave support to a study for detecting genetic erosion using allozyme and microsatellite analysis on three species of Indian major carps, *Catla catla*, *Cirrhinus cirrhosus (C. mrigala)* and *Labeo rohita*.

The study was the result of increasing concern over a declining growth performance of the three Indian major carps in aquaculture. A former study by Eknath and Doyle (1990) suggested that aquaculture rearing practices might lead to a high level of inbreeding. However, a 10 per cent incidence of hybrids was found in a pond based on analysis of DNA markers (Padhi and Mandal 1997).

The aim of this study was to analyse broodstocks using allozymes, microsatellites and mitochondrial DNA (mtDNA) in order to detect genetic erosion and later to compare the results

Table 1. List of enzymes used, their abbreviations, E.C. number, the tissue and buffer used for the enzyme and the number of loci revealed.

Enzyme	Abbreviation	E.C. No.	Tissue	Buffer	No. of loci
Aspartate aminotransferase	AAT	2.6.1.1	Muscle	B[2]	2
Adenosine deaminase	ADA	3.5.4.4	Eye	A[1]	1
Adenylate kinase	AK	2.7.4.3	Muscle	B	1
Creatine kinase	CK	2.7.3.2	Muscle	B	1
Esterase	EST	3.1.1...	Eye	B	2
Glucose-6-phosphate isomerase	GPI	5.3.1.9	Muscle	A	2
Glycerol-3-phosphate dehydrogenase	G-3-PDH	1.1.1.8	Muscle	A	1
Iso citrate dehydrogenase	IDH	1.1.1.42	Eye	B	2
Lactate dehydrogenase	LDH	1.1.1.27	Eye	B	2
Malate dehydrogenase	MDH	1.1.1.37	Eye	B	2
Mannose-6-phosphate isomerase	MPI	5.3.1.8	Eye	B	1
Peptidase (leucyl-glycyl-glycin)	PEP(LGG)	3.4.11 or 13	Muscle	C[3]	1
Peptidase (valyl-leucine)	PEP(VL)	3.4.11 or 13	Muscle	C	1
Phosphogluconate dehydrogenase	PGDH	1.1.1.44	Eye	A	1
Phosphoglucomutase	PGM	5.4.2.2	Muscle	B	1
Purine nucleoside phosphorylase	PNP	2.4.2.1	Eye	B	1

[1] Buffer A Clayton and Tretiak (1972)
[2] Buffer B Ayala et al. (1972)
[3] Buffer C Ridgway et al. (1970)

Table 2. Allelic frequencies of 21 loci analysed in three nursery samples of carps. N is the number of individuals scored.

Locus	Allele	*C. catla*	*C. cirrhosus*	*L. rohita*
AAT-1	1	0.97	0.18	0.80
	3	0.03	0.82	0.20
	N	30	30	30
ADA	4	0.35	0.40	0.77
	5		0.60	0.18
	6	0.65		0.05
	N	30	30	30
AK	1	1.00	1.00	1.00
	N	30	30	30
CK	2	0.97	0.18	0.75
	3	0.03	0.75	0.25
	4		0.07	
	N	30	30	30
EST-1	2		0.50	0.18
	3	1.00	0.50	0.82
	N	30	30	30
EST-2	2	0.63		0.05
	3		0.82	0.18
	4	0.37	0.18	0.77
	N	30	30	30
GPI-1	1	0.97	0.18	0.80
	2		0.23	0.07
	3	0.03	0.58	0.13
	N	30	30	30
GPI-2	2	0.97	0.18	0.80
	4	0.03	0.82	0.20
	N	30	30	30
GPD	2	0.98	0.80	1.00
	3	0.02	0.20	
	N	30	30	30
IDH-1	1	0.15	0.87	0.52
	2	0.85	0.13	0.48
	N	30	30	30
IDH-2	1	1.00	1.00	1.00
	N	30	30	30
LDH-1	1	1.00	1.00	1.00
	N	30	30	30
LDH-2	1	1.00	1.00	1.00
	N	30	30	30
MDH-1	1	1.00	1.00	1.00
	N	30	30	30
MDH-2	1	1.00	1.00	1.00
	N	30	30	30
MPI	1	0.97	0.18	0.82
	3	0.03	0.80	0.18
	5		0.02	
	N	30	30	30
PEP(LGG)	2	0.12	0.02	0.32
	3	0.87	0.17	0.50
	4	0.02	0.72	0.18
	5		0.10	
	N	30	30	30
PEP(VL)	2	1.00	0.95	0.93
	3		0.05	0.07
	N	30	30	30
PGM	1	0.68	0.82	0.38
	2	0.02		0.03
	3	0.27	0.18	0.58
	4	0.03		
	N	30	30	30
PGD	1	0.63		0.05
	2		0.82	0.20
	3	0.37	0.18	0.75
	N	30	30	30
PNP	1	0.70		0.03
	2		0.75	0.17
	3	0.30	0.25	0.75
	4			0.05
	N	30	30	24

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to wild samples from rivers. As it was not possible to get samples from broodstocks, the study was based on the analyses of fingerlings from nurseries. This paper presents the results of the analysis by allozyme electrophoresis of sample fingerlings of the three species of Indian major carps.

Materials and Methods

Thirty individuals of each species (fingerling size, 4 to 8 cm) were obtained from a local middleman. The fish were brought live to the laboratory at Bangladesh Agricultural University and morphologically identified to species with help of the suppliers and the staff at the university. A muscle sample was removed from each fish and stored in absolute ethanol. The rest of the fish was put into a small plastic-bag and, together with the muscle sample, placed in a freezer at – 20°C. The samples were brought to the National Environmental Research Institute (DMU), Silkeborg, Denmark for analyses of allozymes and to the Danish Institute for Fisheries Research (DFU), Silkeborg, Denmark for analyses of microsatellites and mtDNA.

Sixteen enzymes could be reliably scored, representing 21 loci (Table 1). The buffers used were listed in Table 1 and the staining procedures used were similar to the ones described by Machenko (1994).

The data was analysed using the software G-stat (Siegismund 1995), a general software for population genetic analyses, and Structure (Pritchard et al. 2000). Structure is a novel approach for assigning individuals to populations and, in the present case, to species. In addition to assignment tests, it also allows for estimating individual admixture proportions, i.e., the proportion of an individual's genome derived from one or the other population or species. Finally, factorial correspondence analysis was used to analyse the genetic relationships among individuals. This analysis was conducted using the software Genetix 4.0 (Belkhir 1998).

Results and Discussion

Allelic frequencies are listed in Table 2 and various measures of genetic variation are presented in Table 3. Tests for fit to Hardy-Weinberg expectations were performed for 14 loci from *C. catla* and no significant deviations were found. However, 1 of 15 polymorphic loci in *C. cirrhosus* and 6 of 14 polymorphic loci in *L. rohita* showed significant deviations after Bonferroni correction (Rice 1989). The significant deviations were all due to lack of heterozygotes. Depicting the individual heterozygosity, a bimodal distribution was seen for *C. cirrhosus* and lesser pronounced for *L. rohita* (Fig. 1, upper part). An example of a theoretical distribution was shown in

Table 3. Genetic variation in three samples of Indian carps. A locus is defined as polymorphic if the allelic frequency of the most common allele is 0.99 or less (P_{99}).

Sample	Number of loci (n)	Average number of individuals scored (N)	Fraction of polymorphic loci (P_{99})	Average number of alleles (A)	Average observed heterozygosity (H_o)	Average expected heterozygosity (H_e)
C. catla	21	30.00 ± 0.00	0.67	1.81 ± 0.75	0.11 ± 0.12	0.15 ± 0.19
C. cirrhosus	21	30.00 ± 0.00	0.71	1.95 ± 0.81	0.26 ± 0.22	0.25 ± 0.19
L. rohita	21	30.00 ± 0.00	0.67	2.05 ± 0.92	0.15 ± 0.14	0.26 ± 0.21

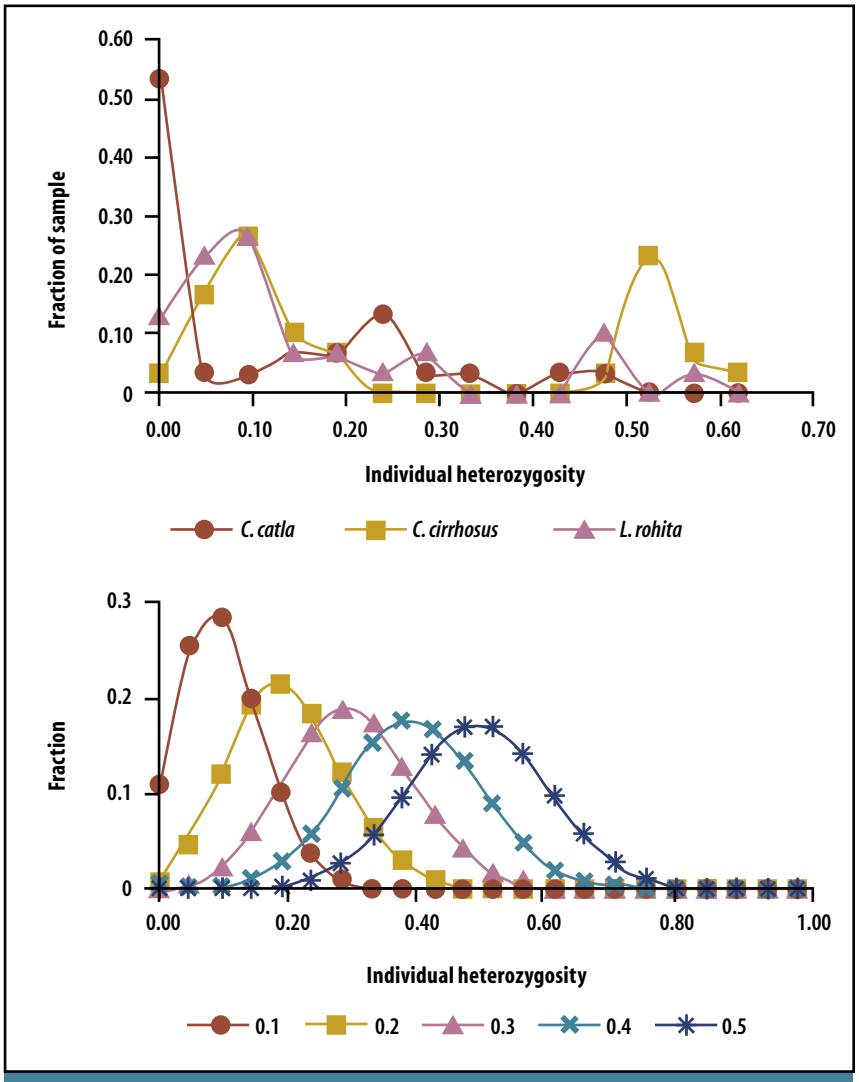

Fig. 1. Distribution of individual heterozygosity for 21 loci in three samples of Indian major carps (upper part) and the hypothetical distribution of individual heterozygosity (lower part), assuming identical frequency of heterozygotes for all loci.

Fig. 1 (lower part). The excess of heterozygotes in the *C. cirrhosus* sample might be due to hybrids between species. The deficiency of heterozygotes in the *L. rohita* sample might be caused by two reproductively separated populations or species (i.e., other species incorrectly identified as *L. rohita*).

The results of the analysis of individual admixture proportions using the software Structure are shown in Fig. 2. It is apparent that the *C. catla* sample contained three fish that were clearly "pure" *L. rohita*, despite having been classified as *C. catla* based on morphological characteristics. Two fish appeared to be *L. rohita* x *C. cirrhosus* hybrids, and 10 fish were hybrids between *C. catla* and *L. rohita*. In the sample of presumptive *C. cirrhosus*, 11 fish were hybrids between *C. cirrhosus* and *L. rohita*. This high incidence of hybrids might explain the high number of allozymes with excess of heterozygotes, as mentioned above. In the *L. rohita* sample, three individuals were most likely *C. cirrhosus*, which might have caused the observed deficiency of heterozygotes, but it was not compensated by the three *C. catla* x *L. rohita* hybrids and four *C. cirrhosus* x *L. rohita* hybrids.

The visualization of genetic relationships among individuals using factorial correspondence analysis (Fig. 3) confirmed the analyses of individual admixture proportions. Three groups of "pure" individuals of the species were identified, along with some individuals that had been misclassified to species. Additionally, several individuals exhibited an intermediate position relative to the parental species and are presumably hybrids, again corresponding to the results of the individual admixture proportion analyses.

The conclusion of this study is that all the three species can interbreed, as mentioned in the review by Das et al. (1996). However, in addition to F_1 hybrids, F_2 or backcrosses were probably also found in the nursery sample (see fish No. HC14, HC25, HR28 and HR26 in Fig. 2). The presence of *L. rohita* in the *C. catla* sample and *C. cirrhosus* in the *L. rohita* sample further illustrated the difficulties in species identification based exclusively on morphological characteristics at the fingerling stage.

These results point to the possibility of hybridisation being at least partly responsible for the decreased growth performance of these species in aquaculture. Thus, Gopal et al. (1989) report slower growth of *C. catla* x *C. cirrhosus* hybrids compared to the parental species, *C. catla*. Therefore, it has to be emphasised that broodstock fish have to be pure species to avoid further genetic erosion of the three species, *C. catla*, *C. cirrhosus* and *L. rohita*.

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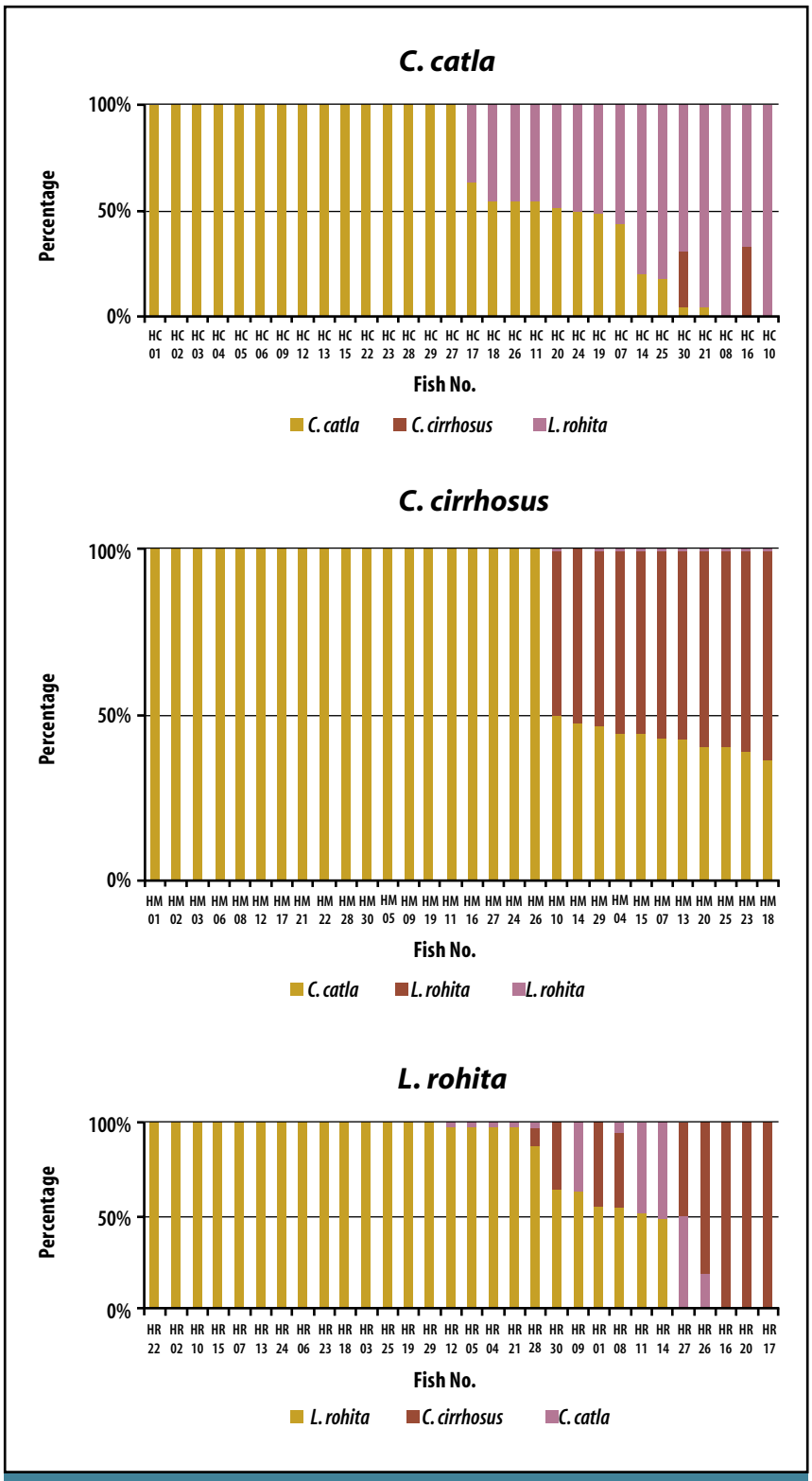

Fig. 2. Results of analyses of individual admixture proportions using Structure (Pritchard et al. 2000). The figure shows the proportion of the genome of each individual in the three samples of putative *C. catla*, *C. cirrhosus* and *L. rohita*, estimated to be derived from any of the three species.

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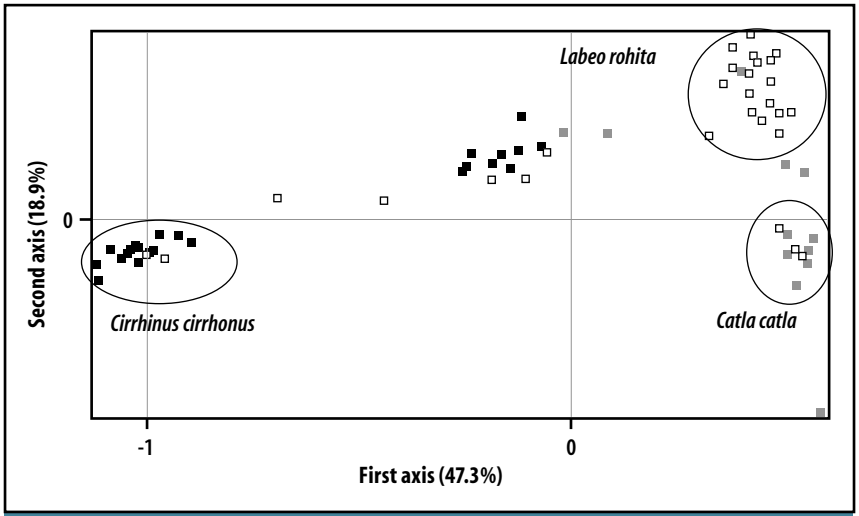

Fig. 3. Factorial correspondence analysis plot showing the genetic relationships among individuals from the three putative species. Black squares denote individuals visually identified as *C. cirrhosus*, white squares denote *L. rohita*, and grey squares denote *C. catla*. Some individuals exhibit identical genotypes, therefore the number of squares does not add up to the total sample size (90). The approximate locations of "pure" individuals of the three species are indicated by circles.

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