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Random Amplified Polymorphic DNA Finger Print and Genetic Similarity Among Four Genera and Between Two Phenotypes of Cultured Carps in Egypt

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ABSTRACT

The random amplified polymorphic DNA (RAPD) assay was evaluated for studying genetic similarity and diversities in four genera and two phenotypes of carps in Egypt. Thirteen random primers were used. The results showed closer proximity of silver carp, *Hypophthalmichthys molitrix* to bighead carp, *Aristichthys nobilis* (76.7%), grass carp, *Ctenopharyngodon idella* (61.7%) and scaly common carp, *Cyprinus carpio* (60.4%). The phylogenetic analysis demonstrated that silver carp is the closest to bighead carp and the farthest from scaly carp. The highest genetic similarity exhibited was between the two phenotypes of common carp, scaly carp and mirror carp (82.0%).

Key Words: Carps; Genera; Genetic similarity; RAPD-PCR; Finger printing

INTRODUCTION

Carps are the most commonly cultured group of fishes in the world (Billard *et al*., 1995; Hulata, 1995; El-Zaeem, 1996; Bartfai *et al*., 2003) and taxonomically belong to the *Cyprinidae,* the largest family among freshwater teleosts (Nelson, 1994), for which the world's annual total catch in 1999 was estimated above 15.6 million metric tons, compared to the 2.3 million tons of salmonids (FAO, 2001). Carps include four primary species in Egypt: common carp *(Cyprinus carpio)*; grass carp *(Ctenopharyngodon idella)*; silver carp *(Hypophthalmichthys molitrix)* and bighead carp (*Aristichthys nobilis*). The last three species commonly are referred to as Chinese carps (Jhingran & Pullin, 1985; Issa, 1987; Hulata, 1995; El-Zaeem, 1996).

Common carps are believed to have originated in central Eurasia (Balon, 1974; Nelson, 1984). Huet (1986) divided this species into four principal phenotypes with different scales cover. The scaly carp (Schuppenkarpfen) is completely covered with scales, the mirror carp (Spiegelkarpfin) has scales of different sizes on its sides and generally one row of scales high on its back, carp with a single row of scales (Zeilkarpfen) have the row on their sides (it may be complete or incomplete, but the scales are of identical size), they also have a few scales at the base of the fins and on top of the back, and leather carp (Nacktkarpfen) have very few scales found generally at the base of the fins. In the light of the genetic constitution of common carp, it is not possible to raise a pure type of carp with one row of scales, or naked carp (heterozygotes), while it is possible to obtain pure lines of carp with scales and also mirror carp (homozygotes). Among the four different types, only carp with scales and mirror carp seem to be suitable for raising (Huet, 1986).

The Chinese carp occur naturally in the major Chinese river systems and the Amur basin in Russia. No distinct varieties or strains of Chinese carps have been documented, nor have genetic differences between populations of different river systems. The domestication of these species was practically prohibited by the dependence of farmers on fry or breeders collected from the wild (Jhingran & Pullin, 1985; Hulata, 1995).

The use of DNA markers can contribute significantly to the development and implementation of genetic improvement programs. Genetic improvement programs in terrestrial species benefit greatly from knowledge of pedigree and individual performance. Properly designed breeding programs can make appropriate use of additive and non-additive genetic variation for traits of economic importance and minimize the negative effects of inbreeding. DNA markers can be used to verify pedigrees, screen wild populations to maximize diversity in founder animals, and to monitor inbreeding levels in breeding populations (Moore *et al*., 1999). Additionally, markers can be used to characterize quantitative trait loci (QTL) (Andersson *et al*., 1994), thereby enabling marker-assisted selection to be applied as an additional component to a selective breeding program. There are, thus, compelling reasons for the

development of DNA markers for use in carp breeding.

Reports have only been published recently on carp genotypes using random amplified polymorphic DNA (RAPD) (Dong & Zhou, 1998; Zhou *et al*., 2001; Bartfai *et al*., 2003; Barman *et al*., 2003) and micro-satellite markers (Crooijmans *et al*., 1997; Aliah *et al*., 1999; Tanck *et al*., 2000, 2001; Desvignes *et al*., 2001; David *et al*., 2001; Lehoczky *et al*., 2002; Bartfai *et al*., 2003). RAPD analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh & McClelland, 1990; Williams *et al*., 1990). The method is simple and quick to perform, and most importantly, no prior knowledge of the genetic make-up of the organism in question is required (Hadrys *et al*., 1992). This technique has been used extensively to detect genetic diversity in plants (Williams *et al*., 1993), animals (Cushwa & Medrano, 1996) and microbes (Carretto & Marone, 1995). RAPD analysis also has been used to evaluate genetic diversity for species and subspecies identification in guppy (Dinesh *et al*., 1993), tilapia (Bardakci & Skibinski, 1994; Dinesh *et al*., 1996), brown trout and Atlantic salmon (Elo *et al*., 1997), largemouth bass (Williams *et al*., 1998) ictalurid catfishes (Liu *et al*., 1998), seabass, seabream and mullet (Ali *et al*., 2004).

The specific objectives of the present study were to estimate genetic similarity among the four genera of cultured carps (*Cyprinus, Ctenopharyngodon, Aristichthys, & Hypophthalmichthys*) and between two phenotypes of common carp (*Cyprinus carpio),* scaly carp and mirror carp in Egypt.

MATERIALS AND METHODS

The present study was carried out at the Nucleic Acid Research Department, (GEBRI), Mubarak City for Scientific Research and Technology Applications, and Animals and Fish Production Department, Faculty of Agriculture, Saba-Bacha, Alexandria University, Alexandria, Egypt.

Species and phenotypes studied. Species and phenotypes of family *Cyprinidae* used in this study and their source have been mentioned in Table I.

DNA extraction. DNA was extracted from fine tissue (liver) of each genus following the method described by Bardakci and Skibinski (1994). Approximately 50 mg of the liver tissue (bulk of 5 fishes) was cut into small pieces and suspended in 500 µl STE (0.1 M NaCl, 0.05 M Tris & 0.01 M EDTA, pH 8). After adding 30 µl 10% SDS and 30 µl proteinase K (10 mg ml^{-1}). The mixture was incubated at 50°C for 30 min. DNA was purified by successive extraction with phenol: chloroform: isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1), respectively. DNA was precipitated with ice-cold absolute ethanol and washed with 70% ethanol. The pellet was dried and resuspended in 200 µl water.

PCR Primers. In the present study, ten and twenty base long oligonucleiotide primers were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification.

PCR amplification and agarose gel electrophoresis. PCR amplifications were performed following the procedures of Williams *et al*. (1990 & 1993) The reaction (25 µl) was carried out in a mixture consisting of 0.8 U of *Taq* DNA polymerase (Fanzyme), 25 pmol dNTPs, and 25 pmol of random primer, 2.5 µl. 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Ependorf). The PCR programme included an initial denaturation step at 94°C for 2 min, followed by 45 cycles with 94°C for 30 sec for DNA denaturation, annealing as mentioned with each primer in Table II, extension at 72°C for 30 sec and final extension at 72°C for 10 min. The samples were cooled at 4°C. Approximately 10 µl of PCR products were resolved on 2% agarose gels by submarine gel electrophoresis in 0.5 X TBE buffer. Subsequently, gels were stained with ethidium bromide and photographed on a UV transilluminator.

Scoring and analysis of RAPDs. The DNA bands were scored for their presence (1) or absence (0) in the RAPD profiles. The band sharing index of similarity between the two phenotypes of common carp was calculated using the formula: $B_{ab} = 2 N_{ab} / (N_a + N_b)$, where N_{ab} is the number of common fragments observed in individuals a and b, and N_a and N_b are the total number of fragments scored in a and b respectively (Lynch, 1990). With respect to the four genera studied, a dendrogram was constructed using the average linkage between groups. The data matrix so generated was used for calculation of similarity matrix for all primers based on Jaccard's coefficients (Jaccard, 1908).

RESULTS AND DISCUSSION

All the thirteen primers (Table II) examined produced different RAPD fragment patterns. The number of amplified bands detected varied, depending on the primers, species and phenotypes. To ensure that the amplified DNA bands originated from genomic DNA, not from primer artifacts, negative control was carried out for each primer/ species combination. No amplification was detected in the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Fig.1 & 3).

With respect to the four genera of carps, RAPD analysis was used for constructing parsimony tree depicting relationships among the four genera studied (Fig. 2). Data presented in Table III and Fig. 2 showed closer proximity of silver carp to bighead carp (76.7%), grass carp (61.7%) and scaly carp (60.4%). The highest interspecies genetic similarity exhibited between silver carp and bighead carp. Lu *et al*. (1997) reported the same results of the genetic

Table I. Species and phenotypes of family *Cyprinidae* used in this study and their source*

No.	Genus	Species	Common name	Group name
1	*Cyprinus*	*carpio*	Scaly carp Mirror carp	Common carp
2	*Ctenopharyngodon*	*idella*	Grass carp	Chinese carp
3	***Aristichthys***	*nobilis*	Bighead carp	
4	*Hypophthalmichthys*	*molitrix*	Silver carp	

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Table II. The sequences, GC% and the annealing temperatures of the RAPD primers used

Primers	Sequence 5`- 3`	GC%	Annealing Tm °C /Sec
A1*	CAG GCC CTT CCA GCA CCC AC	70	52/30
A7▪	GAA ACG GGT GGT GAT CGC AG	60	
A10▪	GGA CTG GAG TGT GAT CGC AG	60	58/30
B4▪	GGA CTG GAG TGG TGA CGC AG	65	
A9▪	GGT GAC GCA GGG GTA ACG CC	70	54/30
R1*	AGGCCCCTG T	70	28/30
R2*	ATGCCCCTG T	60	
R7▪	ACCGCCGAAG	70	
P5#	GAATGCGACG	60	40/30
P8▪	GGG CTAGGGT	70	
P9*	ACCGGG AACG	70	
P10#	AGCAGGTGGA	60	
P7▪	CTGAGG AGTG	60	45/30

▪primers used to study similarity among genera and between the two phenotypes of common carp.
*primers used to study similarity among four genera only.
#primers used to study similarity between the two phenotypes of common carp.

Table III. Jaccard's similarity coefficients among four genera of cultured carps based on RAPD data of all primers used

Genera	Scaly carp	Grass carp	Bighead carp	Silver carp
Scaled carp	-	52.1	55.3	60.4
Grass carp	-	-	60.0	61.7
Bighead carp	-	-	-	76.7
Silver carp	-	-	-	-

similarity of Chinese carps (silver carp, bighead carp & grass carp) based on mt DNA diversity. Bartfai *et al.* (2003) used RAPD marker to discriminate between two stocks of Hungarian common carp and found that the genetic similarity was high.

The results of genetic similarity among the four genera of cyprinids based on RAPD marker can contribute significantly to the development and implementation of genetic improvement programs. An extensive cross-breeding program among four cyprinids (common carp, bighead carp, silver carp & grass carp) was carried out in Hungary (Bakos *et al*., 1978), aimed at producing hybrids suitable for stocking ponds and natural waters. Most suitable for aquaculture were the two reciprocal crosses between the silver carp and bighead carp, which had higher survival and yield than the parental species (Issa *et al*., 1986). Another cyprinid hybrid of interest was the grass carp-x-bighead carp was reported to be triploid (Marian & Krasznai, 1979; Beck *et al*., 1980; Hulata, 2001). Both reciprocal crosses between grass carp and bighead carp resulted in low viability and gross malformations. The grass carp x-silver carp hybrid was reported with accelerated growth (Hulata, 1995). The common carp-x-silver carp hybrid was viable (but not the reciprocal hybrid). The common carp x grass carp hybrid (but not the reciprocal) also was viable. The common carp-x-bighead carp was viable and had good growth rate in the first and second years, but the reciprocal gross was not attempted (Hulata, 1995). The previous reports of cross breeding program reported by several authors support the findings and reflective of the fact that the genetic similarity based on RAPD marker could be used as a rapid and easy way for predicting the hybridization

Fig. 1. Example of RAPD patterns in four genera of carps obtained with different random primers. Lane M: ΦX174 DNA marker, the lanes (1-4) of each primer are: Genus *Cyprinus, Ctenopharyngodon, Aristichthys* and *Hypophthalmichthys,* respectively

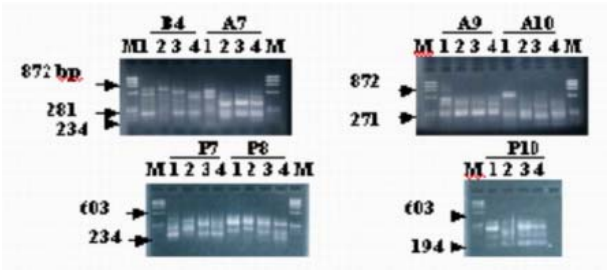

Fig. 2. Dendrogram using Average Linkage (Between Groups) based on RAPD data analysis among the four genera used in this study

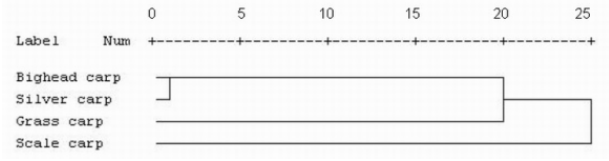

Fig. 3. Example of RAPD patterns in two phenotypes of common carp obtained with different random primers. Lane M: ΦX174 DNA marker. The lanes (1-2) of each primer are: scaly carp and mirror carp, respectively

success among the different genera and species of cultured carps.

The genetic similarity between the two phenotypes of common carp was calculated as band sharing (BS) from RAPD profiles (Fig. 3). The results showed that there is great genetic similarity (82.0%) between the two phenotypes of common carps; scaly carp and mirror carp. The high similarity obtained may be due to the respective forms being rarely phenotypic variants.

This study reports on the use of RAPD markers for studying genetic similarity among the four genera of cultured carps in Egypt for the fist time. The RAPD assay has been used to construct phylogenetic trees for resolving taxonomic problems in many organisms (Chalmers *et al*., 1992; Bardakci & Skibinski, 1994; Greef & Triest, 1999; Barman *et al*., 2003; Soliman *et al*., 2003; Ali, 2003). Nevertheless, the specific characteristics of the RAPD method (random, un-characterized multiple genome loci; dominant nature of markers; and possibility of comigrating, nonhomologous bands) result in limitations based on RAPD analysis alone. Despite these limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species (Barman *et al*., 2003). This work represents a first step towards the generation of DNA markers for purposes, such as species diagnosis, detection of molecular markers linked to economic traits, assessment of genetic diversity and studies on molecular systematic.

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