Genetic variation among four catfish species of family *Bagridae* as revealed by RAPD-PCR assay

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ABSTRACT

Catfishes (order Siluriformes) include about 31 families and 400 genera comprising together over 2200 described species. Bagrid catfishes (family Bagridae) are among the well marketable freshwater fishes in Egypt, because of their affordability and tasteful flesh.

Random amplified polymorphic DNA (RAPD) analysis was applied to four species belonging to family Bagridea: Bagrus bayad, Bagrus docmac, Chrysichthys auratus and Chrysichthys rueppelli, that are widely distributed in the whole River Nile and Naser lake.

To estimate RAPD variations among these four species, genomic DNA samples were examined using 10-mer arbitrary primers. Among the ten primers tested, six produced highly clean and reproducible RAPD profiles. A total of 150 bands were amplified from the ten primers. Out of these, 15 showed common bands which were shared by the four investigated species, the other 135 bands (90%) were polymorphic. Individual primers differed in the amount of interspecific variation detected. On average, each primer produced 13.5 polymorphic fragments. The results were presented in the form of a matrix of presence-absence data for the 150 RAPD bands, and Jaccard's coefficients of similarity were used to generate a dendrogram illustrating the genetic similarity among the four species analyzed.

In this study, RAPD analysis has generated DNA markers that can distinguish clearly the four investigated species.

Key words: *RAPD-PCR, Bagrid catfish, genetic variation.*

INTRODUCTION

Recently, molecular marker techniques, such as RFLP, RAPD and AFLP, have provided tools to evaluate genetic variation and explore evolutionary relationships among and within species (Baradakci and Skibinski 1994; Li *et al.*, 2001). Random amplified polymorphic DNA (RAPD) technique (Williams *et al.*, 1990) is based on the poylmerase chain reaction (PCR) amplification of genomic DNA with single primers of arbitrary nucleotide sequences. These primers detect polymorphism in nucleotide sequences that can be used as genetic markers.

Genetic analysis with RAPD markers is relatively easy, fast and efficient. Therefore, RAPD markers have been widely used for evaluating genetic variation and genetic relationship in plants (Tatineni *et al.*, 1996; Chen and Sun, 1997), insects (Hadrys *et al.*, 1992), and mammalian populations (Woodward *et al.*, 1992). The RAPD analysis

has also been used for the detection of genetic variation in various fish species (Foo *et al.*, 1995; Cunningham and Mo, 1997).

Bagrid catfish (family *Bagridae*) is among the well marketable freshwater fish in Egypt because of its affordability, tasteful flesh and relative abundance in comparison with other fishes. They are widely distributed in the whole river Nile and Naser lake.

The major objectives of the present study are: (1) to evaluate genetic variation among four species of Bagrid catfish using RAPD markers, and (2) to establish genetic relationships among these four species.

MATERIALS AND METHODS

Experimental fish

Fishes used in this study were collected from the river Nile at Sohage (Upper Egypt). They were kept in aquaria until arrival at the Genetics Department Laboratory, Assiut University. Four Bagrid catfish species, namely *Bagrus bayad* and *B. docmak* of the genus Bagrus, and *Chrysichthys auratus* and *Ch. rueppelli* of the genus Chrysichthys, were investigated. Samples of 3 to 5 fishes of each species were selected to estimate RAPD variations between species.

Genomic DNA extraction

The fishes were placed on ice for 16 hr before liver tissue was collected. Tissue was transferred into liquid nitrogen and stored in a freezer until processed for RAPD analysis. Genomic DNA was prepared from the liver tissue using standard protocol (Strauss, 1989; and Liu *et al.*, 1998) with minor modifications. Approximately, 50 mg of the liver tissue was cut into small pieces and suspended in 500 µl STE (0.1 M Na_2 Cl, 0.05 M Tris and 0.01 M EDTA, pH 8). Thirty µl sodium dodecyl sulfate (10%) and 30 µl proteinase K (10 mg/ml) were added and the mixture was incubated at 50° C for 12 hrs. DNA was purified by successive extractions with phenol: chloroform : isoamyl alcohol (25 : 24 : 1) and chloroform : isoamyl alcohol (24 : 1). DNA was precipitated with ice-cold absolute ethanol and then washed with 70% ethanol. The pellet was then dried and resuspended in 150 µl TE buffer (10 mM Tris-HCl, 1 mM Na EDTA-H_2O, pH 7.2).

PCR reaction

The protocol described by Williams *et al.* (1990) was employed for RAPD analysis with 10 decamer primers from Operon Technologies Inc. (Alameda, CA) (Table 1). The optimum PCR reaction mixture was a total of 25 ml containing 20 ng of genomic DNA; 100 µM each of dATP, dTTP, dGTP and dCTP; 2.5 mM Mg Cl_2; 5 pmoles of a single decamer primer and 0.5 units of Taq DNA poylmerase (GIBCO). Each reaction mixture was overlead with one drop of mineral oil. DNA amplification was performed in a thermal cycler (Perkin Elmer, Cetus, Norwalk, CT). Each sample was run for 2 replications. The thermal cycles used were: one cycle of 3 min at 94° C, 2 min at 45° C and 2 min at 72° C; 35 cycles of 1 min at 94° C, 1 min at 37° C and 2 min at 72° C and a final extension at 72° C for 7 min, followed by a slow cooling to 4° C. The amplified products were subjected to electrophoresis in 1.4 % agarose gel. The gels were stained with ethidium bromide and photographed under ultraviolet light. *Lambda* phage DNA digested with Hind III and Φ *X*-174 DNA digested with *Hae* III were used as standard DNA marker.

Data analysis

Amplified products were scored on the basis of their presence or absence. Only data generated from the detection of clear and stable amplified fragments were analyzed. Genetic similarity (GS) values were obtained

following Jaccard (1908). In this method, each pairwise comparison gives a value of 1 for a similarity and 0 for a difference, and the genetic similarity is equal to the numerical mean of the set of observations. The unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) was used to generate a dendrogram based on the GS matrix.

RESULTS

Genetic variation

A total of 150 DNA fragments were amplified by 10 decamer primers with an average of 15 fragments per primer across all material (Table 1). The number of products amplified by each primer varied from 8 with primer OPZ-13 to 24 with primer OPB-10. Among the 150 amplified fragments, 135 were polymorphic with an average of 13.5 per primer, ranging from 8 for primer OPZ-13 to 20 for primer OPZ-06, respectively (Table 1). All 10 primers produced polymorphic fragments and the polymorphism for individual primers varied from 100% with OPA-17, OPO-03 and OPZ-13 to 75% with OPB-10. Of the fragments, 90% were polymorphic, indicating that there is a considerable variation at the DNA level in the species investigated.

Some primers generated species-specific fragments, while monomorphic fragments were also detected (Fig. 1). It was observed that 45 fragments were unique to different species and represent specific markers,. The specific fragments 290, 460, 890 and 2000 bp were only found in *Ch. auratus*, while fragments 610, 710, 920, 1050 and 2500 bp were only present in *B. docmak*. These unique amplified products may be used to identify the two species.

Genetic similarity

Based on RAPD analysis, pairwise genetic similarities (GS) were estimated and ranged from 0.131 to 0.747 among the four investigated species (Table 2). Out of 6 pairs of species combinations, four had a GS of less than 0.40, indicating a wide genetic difference between them, i.e., *between* the species of the two genera. However, GS in 2 pairs of

Table (1): Primer code, nucleotide sequence, amplified products, and polymorphism percentage in the four Bagrid fish species investigated.

Primer code	Nucleotide *to*	Amplified bands	Polymorphic bands	Polymorphism[1]
OPA-10	GTGATCGCAG	10	9	90
OPA-17	GACCGCTTGT	15	15	100
OPA-20	GTTGCGATCC	12	10	83.3
OPB-10	CTGCTGGGAC	24	18	75
OPC-05	GATGACCGCC	13	12	92.3
OPC- 07	GTCCCGACGA	17	15	88.2
OPE-07	AGATGCAGCC	16	14	87.5
OPO-03	CTGTTGCTAC	14	14	100
OPZ-06	GTGCCGTTCA	21	20	95.2
OPZ-13	GACTAAGCCC	8	8	100
Total		150	135	90
Average		15	13.5	

[1]Polymorphism = (polymorphic bands / amplified bands) * 100.

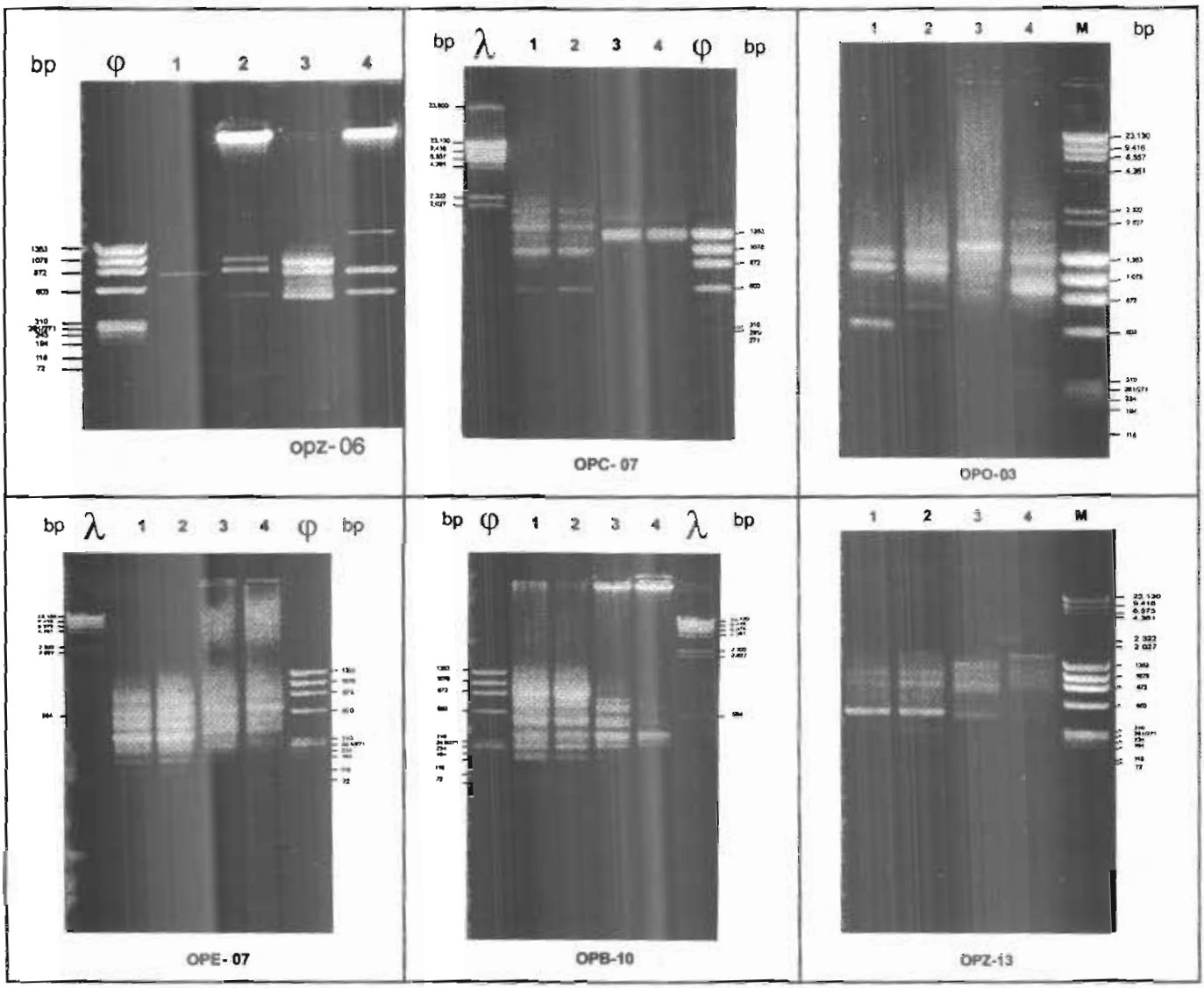

Fig. (1): Agarose-gel electrophoresis of RAPD products generated with six decemar arbitrary primers in four species of Bagride catfish.
1- Chrysichthys auratus 2- Ch. ruppelli 3- Bagrus bayad 4- B. docmak. - ve negative control. M = Marker.

Table (2): Absolute Jaccard's similarity (GS) coefficient matrix.

Species	*C. auratus*	*C. rueppli*	*B. bayad*	*B. docmak*
C. auratus				
C. rueppli	0.7470			
B. bayad	0.1308	0.1515		
B. docmak	0.1491	0.2143	0.4681	

combinations were greater than 0.40 such as *Ch. auratus* and *Ch. rueppIli* (0.747) and *B.bayad* and *B. docmak* (0.468), showing that they have a close genetic relationship.

Cluster analysis:

A dendrogram indicating genetic relationships between the four investigated species was generated by cluster analysis (UPGMA) with the RAPD data (Fig. 2). The four species were classified into two groups. The first group includes the two species of the genus *Chrysichthys*, while the second group contains the two species of the genus *Bagrus*. These results showed that the classification by RAPD data reflected existing differences between different species. However, these differences are larger between the two groups than within the groups.

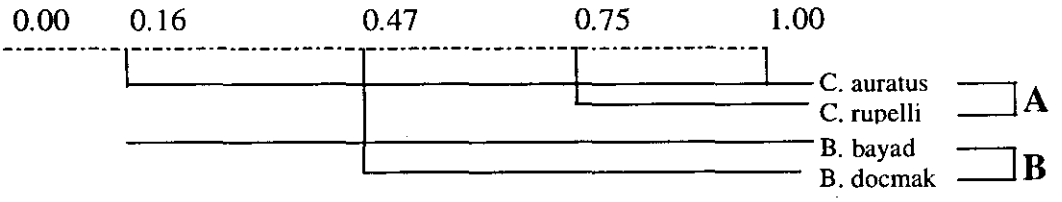

Fig. (2): Dendrogram generated for 4 species from 135 polymorphic RAPD markers by cluster (UPGMA) analysis.

DISCUSSION

The obtained results showed the possibility of using RAPD as a precise identification technique of fish species which is of prime importance to clarify the taxonomic position of such species in Egypt, as a pre-step for rearing, artificial spawning and hybridization required for fish farming and breeding. Such possibility is confirmed by the findings of Williams *et al.* (1998) who showed that RAPD analysis has proven itself as an easy tool for identification of largemouth bass subspecies.

Species classification has been largely based on variation in dentition, bone structure, pigmentation, squamation characteristics and general body morphology (Kornfield, *et al.* 1979; Stiassny, 1991; 1992; Trewaves, 1982). However, most or all of these characters overlap and may fail to unambiguously identify species owing to interpopulation variation and small differences among species (Mwanja *et al.*, 1994). To overcome this obstacle, molecular techniques have been employed in an attempt to characterize and identify Tilapine species (Frank *et al.*, 1992; Seyoum and Kornfield, 1992).

The RAPD technique is reliable and simple to apply, proving to be cost effective and appropriate for species identification. The RAPD technique was used by Bardakci and Skibinski (1994) to differentiate among species and subspecies of the Nile Tilapia and three other species of the genus Orochromis in aquaculture.

In the present investigation, RAPD generated a large number of polymorphic DNA bands between the four studied species. Such RAPD markers in the present study are confirmed by the results of Liu *et al.* (1999) in channel and blue catfish.

The extent of genetic variation between four species of family *Bagridae* common in upper River Nile and Naser lake, Egypt, have been

explored in this study. Using ten arbitrary PCR primers, species-specific genetic markers were generated that can be used in the identification of the four investigated species as well as providing taxonomic insight. The RAPD-PCR technique revealed substantial nuclear genomic variation between the four species. This study represents the first application of RAPD technique to the assessment of variation between species of the Bagrid catfish.

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الملخص العربي

التباين الوراثي في أربعة أنواع من أسماك العائلة البقرية باستخدام تقنية الدنا متعدد المظاهر المتعاظم عشوائيا (RAPD)

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تضم أسماك catfish نحو ٣١ عائلة و ٤٠٠ جنسا تمثل معا اكثر من ٢٢٠٠ نوعا. وتعتبر أسماك العائلة البقرية من أفضل الأسماك النيلية من الناحية التسويقية في مصر لرخص ثمنها وجودة مذاق لحمها.

استخدمت تقنية الدنا متعدد المظاهر المتعاظم عشوائيا (RAPD) على أربعة أنواع تابعة للعائلة البقرية هى *Bagrus bayad* و *Bagrus docmak* و *Chrysichthys auratus* و *Chrysichathys ruppelli* وهى من الأنواع واسعة الانتشار فى مياه نهر النيل وبحيرة ناصر.

ولتقدير إختلاف الـRAPD بين الأنواع الأربعة اختبر الدنا الجينومى باستخدام بادئات بكل منها عشرة قواعد، وقد أعطت ست بادئات من العشرة المستخدمة صورا على درجة كبيرة من الوضوح والثبات. وأنتجت البادئات العشر ١٥٠ حزمة منها ١٥ حزمة مشتركة وحيدة المظهر بين الأنواع الأربعة المدروسة بينما كانت باقي الحزم (١٣٥حزمة) متعددة المظاهر بنسبة ٩٠%. وقد تفاوتت البادئات المختلفة فى التباين الذي يمكن اكتشافه بواسطتها بين الأنواع. وقد انتج كل بادئ فى المتوسط ٥و١٣ شظية متباينة . وتم عرض نتائج الدراسة فى صورة مصفوفة لوجود وغياب كل من المائة والخمسين شظية. واستخدم معامل "جاكارد" للتماثل الوراثى لرسم شجرة توضح التماثل الوراثى بين الأنواع الأربعة المدروسة. وقد أعطى تحليل RAPD واسمات دنا جزيئية مميزة لكل نوع بحيث يمكن استخدامها فى التفريق بين الأنواع الأربعة.