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Genetic Diversity of Rice Field Eel (*Monopterus albus*) in China Based on RAPD Analysis

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Abstract

The genetic diversity of seven populations of *Monopterus albus* from China, i.e., Yancheng, Mianyang, Baoding, Suqian, Anshun, Shaoyang and Nanning, was studied based on RAPD analysis. Thirteen of 50 arbitrate primers were screened to detect 122 polymorphic loci in 72 individuals. Shannon index, Nei's gene diversity coefficient and percentage of polymorphic loci analysis consistently indicated that Mianyang and Yancheng populations displayed the largest diversity information, followed by Baoding, Suqian, Nanning, Anshun and Shaoyang in order. The genetic variations were found partitioned mainly within rather than among populations, as the latter accounted for only a small portion of variations (27.9% by AMOVA). Genetic differentiation existed among all the populations (Gst = 0.1798), with a gene flow of 2.2813.The overall Shannon index and Nei's gene diversity index was 0.4991 and 0.3302 respectively. Inferred from genetic distance, a phylogenetic dendrogram was also constructed by UPGMA method for the seven populations. Generally speaking, low genetic diversity was shown for all these populations of *M. albus* in China mainland.

Introduction

Rice field eel, *Monopterus albus,* a Synbranchiformes freshwater fish usually inhabited in subtropical and tropical areas in Asia, is widely cultivated across China in the past decade. It is one of the most valuable freshwater fishes for export and domestic consumption in China's fishery industry. However, due to its idiosyncrasy of burrowing, air breathing and sex reversal phenomenon during maturation, large-scale breeding and rearing for this fish are still problematic. Yet, there has been considerable progress in the practice and study of *M. albus* reproduction and artificial culture in China. Previous researches mainly focused on

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physiology, ecology, disease control, cellular and molecular genetics and environmental toxicology of this species (Tao et al. 1993; Liu et al. 2001; Lu et al. 2002; Xu and Su 2003). To date, the study on the genetic resources of rice field eels has not been reported in a geographically broad scale in China mainland except for the comparison of genetic diversity made by He et al. (2004) among *M. albus* from China, *M. cuchia* from Burma, and *M. fossorius* from Indonesia. The knowledge of genetic background of *M. albus*, in particular, the genetic diversity and genetic differentiation over different regions across the nation is still obscure and urgently required in the aquacultural industry. The objective of this research was to examine the genetic relationships to assess the genetic diversity of *M. albus* from seven geographically widely separated regions in China mainland based on randomly amplified polymorphic DNA (RAPD), expecting to provide a preliminary data for resources conservation and selected breeding of this species.

Materials and Methods

Sample collection

Seventy-two wild individuals of *M. albus* were collected from the rice fields or marsh at seven different sampling regions throughout China in 2002 (Table 1).

The minimum body length was 13 cm and the maximum, 25 cm, with 18 cm in average. Muscle tissues were immediately preserved in 95% ethanol solution after biopsy, then were brought back to laboratory and stored at 4℃ till use.

Table 1. Samples of *Monopterus albus* and their geographic sources

Population name	Sample number, population habitat environment abbreviation and code	Geographic source	Longitude	Latitude
Yancheng/ plain	13;YC, A	Dongtai County, Yancheng City, Jiangsu Province	32º84'N	120º31'E
Mianyang/ plain	8; MY, B	An County, Mianyang City, Sichuan Province	31º64'N	104º41'E
Baoding/ plain	12; BD, C	Gaoyang County, Baoding City, Hebei Province	38º68'N	115º78'E
Suqian/ plain	10; SQ, D	Siyang County, Suqian City, Jiangsu Province	33º73'N	118º68'E
Anshun/ mountainous	9; AS, E	Puding County, Anshun City, Guizhou Province	26º32'N	105º75'E
Shaoyang/ mountainous	10; SY, F	Dongkou County, Shaoyang City, Hunan Province	27º06'N	110º57'E
Nanning/ mountainous	10; NN, G	Shanglin County, Nanning City, Guangxi Province	23º44'N	108º59'E

DNA extraction

Between 100 and 150 mg muscle tissue was minced to fine powder, then transferred to a 1.5 ml Eppendorff tube containing 500 µl lysis buffer (25µl 0.5mol•l^{-1} Tris-HCl, pH8.0; 100µl 0.25mol•l^{-1} ETDA; 50µl 20% SDS; 10µl 20mg•ml^{-1} Proteinase K (Merck Inc.); 10µl 10mg•ml^{-1} RNase; 305µl ddH2O), from which total DNA was extracted. The procedure of DNA isolation and purification were referred to the previous method used in our lab (Qiu and Chang 2001).

Polymerase chain reaction

Fifty random 10-mer primers (Sangon Co., Shanghai, China) of S_1 to S_{50} were used to amplify. The 25 µl PCR reaction mixture was composed of 10 mmol•l^{-1} Tris-HCl pH9.0, 50mmol•l^{-1} KCl, 2.0 mmol•l^{-1} $MgCl_2$, 0.001% glutin, 0.2 mmol•l^{-1} dNTPs, 0.4 µmol•l^{-1} arbitrate primers, 50 ng genomic DNA, and 1.5 U Taq DNA polymerase (Biostar, Canada). Amplification of DNA was performed in a thermal cycler (Eppendorf Mastercycler Gradient). The program was set as: pre-denaturation at 97℃ for 10 min, followed by 40 cycles of 1 min at 94℃; 1 min at 36℃; 1.5 min at 72℃, and a final cycle of 5 min at 72℃. Negative controls without template DNA were run in each reaction.

Electrophoresis and photography

The PCR products were resolved by electrophoresis in 1.6% agarose gels (Sigma Chemicals) for 2 h at 5 V.cm^{-1} .A 100bp DNA ladder (BBST Co., Shanghai, China) was used as size marker. After electrophoresis, gels were stained with ethidium bromide and photographed in a UV light transilluminator (Biostep imaging system, Jahnsdorf, Germany).

Data analysis

The patterns of the electrophoresis resulting from the RAPD PCR products were converted into figure "1" or "0", corresponding to where a clearly defined reproducible band was present or absent. Then these 1, 0 data were fed to RAPDistance v1.04 (Armstrong et al. 1996) to calculate genetic similarity and genetic distance. Standard genetic distances were estimated using Nei's standard genetic distance (Nei 1972) as implemented in the programs. A phylogenetic dendrogram was constructed with unweighted pair group method using arithmetic average (UPGMA) as integrated in MEGA 2.1(Kumar et al. 2001).

The distance matrices were analyzed by WINAMOVA 155 (Excoffier et al. 1992) to define the sources of variation originated from within-population and between-population. To test for significant level of the variations, 9999 permutations were conducted to obtain a P value with Φ statistics as implemented in the program.

Shannon index and Nei's gene diversity index were employed to determine the overall genetic diversity and the degree of genetic divergence among the populations (G*st*) and migration number per generation (Nm). These calculations were made using POPGEN 32 (Yeh and Boyle 1997).

Results

Amplification results of PCR

Thirteen of the 50 arbitrate primers were screened out, which can produce clearly reproducible fragments, to detect 122 polymorphic loci in 72 individuals of seven populations. The codes and sequences of these primers were: S11, 5'-gtagacccgt-3'; S17, 5'-agggaacgag-3'; S22, 5'-tgccgagctg-3'; S28, 5'-gtgacgtagg-3'; S29, 5'-gggtaacgcc-3'; S3, 5'-catccccctg-3'; S31, 5'-caatcgccgt-3'; S38, 5'-aggtgaccgt-3'; S4, 5'-ggactggagt-3'; S45, 5'-tgagcggaca-3'; S6, 5'-tgctctgccc-3'; S7, 5'-ggtgacgcag-3'; and S8, 5'-gtccacacgg-3'.

The fragments amplified by a single primer in all the populations varied from seven to 13, with a molecular weight ranged from 300 bp to 4,000 bp. The percentage of polymorphic

loci differed in seven populations, with the largest 82.79% for populations Yancheng and Mianyang, the smallest 29.51% existing in Shaoyang population (Table 4).

Genetic distance and genetic similarity

Among all the population pairs of *M. albus*, the Nanning -Anshun pair recorded the largest genetic distance of 0.6199 while the Baoding-Suqian pair showed the smallest of 0.0485, with a mean of 0.3505. Table 2 displayed the genetic distances and genetic similarities between and within populations.

The genetic distances within populations scored the biggest for the Mianyang population (0.5130) and the smallest for Anshun population (0.1524), averaging in 0.2811.

Phylogenetic tree

Phylogenetic dendrogram was generated based on Nei's genetic distances for all populations, shown as in Fig. 1.

According to the graph, firstly, the Baoding population groups together with Suqian, then converges with geographically proximate region, Yancheng, followed by clustering with the two southwestern populations, Anshun and Mianyang, which finally gathers to the clades of Shaoyang and Nanning, the two central south populations.

Genetic variation analyzed by AMOVA

The result of analysis of molecular variance was as indicated in Table 3.

This table illustrates the source of genetic variation. Variations were found be partitioned mostly within (61.72%) rather than among populations, as the latter accounted for only a small part of variations (27.90%). And the rest variation (10.38%) originated from among regions, with an unbiased estimate value of 0.104 (P=0.0513).

Table 2. Genetic distances (F*st*) and genetic similarities of *M. albus* among and within populations

	YC	MY	BD	SQ	AS	SY	NN
YC	0.4139	0.8062	0.8924	0.8797	0.7762	0.6083	0.5522
MY	0.1938	0.5130	0.7182	0.6859	0.6272	0.6324	0.6007
BD	0.1076	0.2818	0.2503	0.9515	0.8601	0.5297	0.4617
SQ	0.1203	0.3141	0.0485	0.2284	0.8667	0.5000	0.4549
AS	0.2238	0.3728	0.1399	0.1333	0.1524	0.4407	0.3801
SY	0.3917	0.3676	0.4703	0.5000	0.5593	0.1782	0.4152
NN	0.4478	0.3993	0.5383	0.5451	0.6199	0.5848	0.2314

Notes: Lower triangle matrix are values of genetic distances between populations, upper triangle matrix are values of genetic similarities, and values on diagonal are genetic distances within populations.

Table 3. Analysis of Molecular Variance (AMOVA) for 72 individual *M. albus* of seven populations in three regions, using 122 RAPD marker loci

Sources of variation	df	SSD	MSD	Variance component	% Total	φstatistics	P-value
Among Regions	2	2.6126	1.306	0.02362732	10.38	φCT= 0.104	0.0513
Among Populations/regions	4	3.2098	0.802	0.06353478	27.90	φSC= 0.311	<0.0001
Among Individuals/ populations	65	9.1366	0.141	0.14056342	61.72	φST = 0.383	< 0.0001

Analyses of genetic diversity

Evaluation of genetic diversity was based on levels of single population and all populations.

As can be seen in Table 4, the greatest Shannon index (I) occurred in Mianyang population (0.4568), followed by Yancheng (0.4251), Baoding (0.3515), Suqian (0.3028), Nanning (0.2019) and Anshun (0.1946) in order. Shaoyang population, which only scored 0.1551, was the smallest one. With reference to Nei's gene diversity coefficient (h), the order was Mianyang (0.3071), Yancheng (0.2811), Baoding (0.2352), Suqian (0.2010), Nanning (0.1339), Anshun (0.1287), and Shaoyang (0.1030). The results analyzed by these two parameters were consistent with that by the percentages of polymorphic loci (PL%).

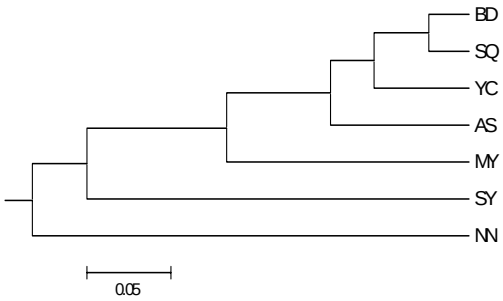

Fig. 1. Phylogenetic dendrogram constructed by UPGMA method for seven populations of *M. albus*, indicating the scale of branch length

Analysis for all of the seven populations of *M. albus* as a whole exhibited that the overall Shannon index was 0.4991, Nei's gene diversity, 0.3302, gene flow among populations, 2.2813, and genetic differentiation index (Gst), 0.1798 (Table 5). These findings revealed that there was a genetic differentiation, albeit low, for the *M. albus* populations throughout China, and the genetic variations mainly came from individuals within populations (82.02%), the remainder contributed by among populations (17.98%).

Table 4. Genetic variations for single population of *M. albus*

	YC	MY	BD	SQ	AS	SY	NN
Sample size	13	8	12	10	9	10	10
h	0.2811	0.3071	0.2352	0.2010	0.1287	0.1030	0.1339
I	0.4251	0.4568	0.3515	0.3028	0.1946	0.1551	0.2019
PL%	82.79	82.79	66.39	57.38	37.70	29.51	38.52

Notes: cf. Table 5

Table 5. Nei's analysis of gene diversity in multi-populations as a whole

Sample size	h	I	Ht	Hs	Gst	Nm
72	0.3302	0.4991	0.3322	0.2725	0.1798	2.2813

Notes: h: Nei's gene diversity; I: Shannon's Information index; Ht: total gene diversity; Hs: gene diversity within population; Gst: coefficient of gene differentiation; Nm: number of migration, the estimate of gene flow; PL%: percentage of polymorphic loci.

Discussion

The results of this study present the genetic differences among geographically isolated populations of *M. albus* based on RAPD data. In general, in terms of most genetic parameters, the populations (Yancheng, Baoding, Suqian) from the maritime provinces (Jiangsu, Hebei, Table 1) showed a relatively high value compared to their southwestern inland counterparts (Anshun, Shaoyang and Nanning), with an exception of Mianyang, which locates within the fertile Sichuan Plain. The possible reasons for this discrepancy will be discussed later in this paper.

Low as it is, the overall Gst value (0.1798, Table 5) revealed that genetic differentiation existed among the *M. albus* populations across China. AMOVA and analysis used by Nei's gene diversity coefficient, despite the different results (61.72% by AMOVA vs. 82.02% by Nei's), demonstrated the same tendency, reflective of genetic variations arising mainly from individuals within populations rather than from among populations(Table 2).This finding differs from the studies of *M. albus* in some states of the U.S., where the genetic diversity within each of the presumed introduced populations was generally low, and was remarkably high among populations (Collins et al. 2002). In comparison with the results of research on the genetic structure of other organisms, such as small yellow croaker (*Pseudosciaena polyactis*) (Meng et al. 2003), squid (*Moroteuthis ingens*) (Sands et al. 2003), tuna (*Thunnus albacares*) (Pindaro and Manuel 2003) and marten (*Martes americana*) (Kyle and Strobeck 2003), our study suggests that, in general, the genetic diversity for the *M. albus* populations in China mainland is not rich enough, though some populations possess a high percentage of polymorphic loci (>80%), the Shannon indices, Nei's gene diversity coefficients are usually low.

As a cavernicolous freshwater species, *M. albus* usually lurks in a crevice or burrow. In the wild, they are confined to a limited habitat, given suitable humidity of milieu and sufficient food supply. Although they are quite capable of using streams for dispersal, migrating to new places for a possible new breeding ground or for a new food source, aside from escaping by means of external elements, such as floods, storms etc., from their wild habitats or ponds, rice field eels (or their gametes) —in natural condition, restricted by the barrier of surroundings—are unlikely to undertake trans-habitat migration over long distances as migratory freshwater eel (*Anguilla sp.*) and some other species of fish that involve both living in freshwater and reproducing in sea water during their life cycles. In this study, an overall gene flow of 2.28 did exist among the seven populations of *M. albus* throughout China, notwithstanding the geographically rather large distances between sampling localities, e.g., the largest between Baoding and Nanning population, 3816 km, and the smallest, 360 km, in Yancheng-Suqian pair, with a mean of 2165 km between any two populations. Therefore, we speculate that, the gene exchange among the different populations of *M. albus* may primarily stem from human activities. Since the 1980s, artificial cultivation and breeding of rice field eels has been carried out in China, especially in the Yangtze River drainage areas of Sichuan Basin, Jianghan Plain, Yangtze Rive Delta, in Pearl River drainage basins and in the coastal provinces. Nowadays, rice field eel has become one of the most important species for the Chinese freshwater fisheries. Trading of rice field eels, e.g., exchange of parental stocks, catching wild juveniles for culture, transporting and selling edible-sized eels, may result in the influence of the genetic structure of *M. albus* between regions.

Anthropogenic interferences can not only enhance the genetic diversity of a specific species, but may also weaken it if improperly governed. For example, pesticide and fertilizer employed in rice agriculture are not well controlled; plant drainage or waste water derived from city life subjected to inadequate treatment prior to discharge, thus, some habitats of rice field eel might be contaminated to an extent. Until recently, the juveniles used in the artificial culture of this fish to adulthood in China are mainly caught in the wild (Zhu 2003). Some underdeveloped regions, especially the mountainous southwestern China, witnessed an overfishing of this species due to inappropriate resources management by the rural government and unrestrained illegal capture by the local peasants, driven by poverty and profits concerns. Obviously, all of these will cause a decline in the natural resources and hence a likely decrease in the genetic diversity of *M. albus* in these regions, provided that such conducts mentioned above are out of control. Either positive or negative, human perturbation would undoubtedly have an impact on the delicate genetic structure of this usually sluggish species,

which has a low fertility, accountable for the overall lowness and the differences of genetic diversity among all of the seven populations. Moreover, this eel is a voracious piscivorous demonstrative of cannibalism—in addition to preying on other organisms, the adults would prey on the juveniles and eggs occasionally in breeding seasons, which would become intense in case of dearth of food and deterioration of their living environment (Li 2001).

Conclusion

In summary, the overall genetic diversity of *M. albus* resources in China mainland was generally low, and a low genetic differentiation occurred among all of the seven populations across the nation. These findings suggest that departments concerned and their decision makers pay more attention to maintain the genetic diversity of rice field eels in China. For a more effective exploitation of this species, it is critical to regulate all of the steps concerning rice field eels catching, rearing, breeding and selling. Only in this way can the sustainable development of *M. albus* be realized and a promising prospect of rice field eel industry in China will unfold ahead of us. In the long run, this will definitely have a far-reaching significance to the freshwater aquaculture in Asia.

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