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ESTIMATION OF GENETIC DIFFERENCES AMONG GUPPIES, *Poecilia reticulata* by RAPD MARKERS

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ABSTRACT

Sixty-four individuals that comprise 8 guppy varieties were used for genetic analysis. Genomic DNA of each individual was extracted from muscle tissues. DNA structure of each sample was examined by using random amplification of polymorphic DNA technique. Nine primers were worked in RAPD-polymerase chain reaction. Seven of them worked well having bands. Genetic similarities and differences for intraspecies and interspecies were calculated. Genetic difference between king and flamingo was the highest (0.604). Genetic similarities of all varieties were determined quietly high (minimum 0.670, maximum 0.948).

Key Words: Guppy, *Poecilia reticulata*, RAPD, genetic variation

Lepistes Varyeteleri, *Poecilia reticulata* Arasındaki Genetik Farklılığın RAPD Markerlar ile İncelenmesi

ÖZET

Sekiz lepistes varyetesi içeren 64 birey genetik analiz için kullanıldı. Genomik DNA kas dokusundan ekstrakte edildi. Her örneğine ait genomik DNA RAPD tekniği kullanılarak incelendi. Dokuz primer RAPD-PCR'da çalıştırıldı. Bu primerlerden sekiz tanesinde band tespit edildi. Varyeteler içi ve varyeteler arası genetik benzerlik ve farklılık hesaplandı. King ve flamingo arasındaki genetik farklılık en yüksek bulundu (0.604). Tüm varyetelerin genetik benzerlikleri oldukça yüksek olarak bulundu (minimum 0.670, maksimum 0.948).

Anahtar Kelimeler: Lepistes, *Poecilia reticulata*, RAPD, genetik farklılık

INTRODUCTION

Guppies (*Poecilia reticulata*) are an important aquarium fish in aquaculture (Alpbaz, 1993 and Khoo et al., 2003). Researchers have constituted many varieties of guppy by crossbreeding among them. They have many different color combinations and body shapes. Some scientists have already studied on their molecular genetics. RAPD that is a common technique have been used to determine guppy genetics (Dinesh et al., 1995; Dinesh et al., 1993; Foo et. al., 1995; Khoo et al., 2002).

Bardakcı and Skibinski (1994) demonstrated genetic differences among three tilapia species and four strains of *Oreochromis niloticus*. Genetic similarity was 0.73 for Nile tilapia, 0.78 for Mozambican tilapia, and 0.87 for Aureus tilapia. Genetic similarity between species was 0.59 for Mozambican/Nile, 0.46 for Aureus/Nile, and 0.38 for Aureus/Mozambican. Dinesh et al. (1995) examined RAPD fragments by agarose gel, continuous polyacrylamide gel electrophoresis (PAGE) and discontinuous polyacrylamide gel electrophoresis (dPAGE) in seven fish species. Foo et al. (1995) investigated inheritance of green snakeskin and black varieties of guppy by RAPD. Parents and F_1 progenies had Mendelian pattern of inheritance. Partis and Wells (1996) used RAPD to find differences among eight species (*Lates calcarifer*, *Lates niloticus*, *Zeus faber*, *Cyttus australis*, *Zenopsis nebulosis*, *Allocyttus verrucosus*, *Neocyttus rhomboidalis*, *Pseudocyttus maculatis*). Bielawski and Pumo (1997) searched genetic variations in Atlantic striped bass (*Morone saxatilis*) by RAPD due to having low variation (heterozygosity 0.32). Caccone et al. (1997) examined DNA polymorphism within population of European sea bass (*Dicentrarchus labrax*) by RAPD. 260 samples were collected from 8 different regions of Mediterranean Sea and the Coast of Atlantic Ocean of Spain. Totally, 107 primers were used for individuals. When high in population, variations were quiet low between populations. It was figured out that band pattern was related with their tolerance to different salinity levels. Genetic variations were searched on offsprings crossbred between salmon fish and non-anadrom brown trout. They were too low and had a monomeric character. Callejas and Ochando (1998) were determined that three native barbus species (*Barbus bocagei*, *Barbus graellsii* and *Barbus sclateri*) morphologically resembled each other. They had 6 bands for *B. bocagei*, 11 bands for *B. graellsii* and 9 bands for *B. sclateri*. It is found that *bocagei* and *graellsii* species had close relation as dendogram. Liu et al. (1998) investigated crossbreeding between channel and blue catfish progenies ($F_1$and F_2). They examined genetic variation of channel catfish and constituted their gene maps Liu et al. (1999). Koh et al. (1999) and Liu et al. (1998) examined discus that is a tropical fish by RAPD. Lehmann et al. (2000) carried on the genetic definition of four eel species (*Anguilla anguilla*, *A. japonica*, *A. reinhardti* and *A. rostrata*) by RAPD. Two species had monomorphic bands that ranged 1-18. Average genetic differences changed 0.384-0.559 among species. Anguilla had minimum difference with Rostrata and maximum with Reinhardti. Any individuals in the same place did not

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have any monomorphic bands. Probably, larvae distributed randomly in ocean waves before reaching any coasts. In dendogram, all Atlantic species took a place in the same group. It is found that Reinhardti could be ancestor of four species. Genetic difference was 0.712 in European species. That of others was 0.531. Khoo et al. (2002) examined genetic diversities of local and domesticated strains in Singapore guppy. Shikano and Taniguchi (2002) investigated heterosis among F_1 progenies crossbred in four guppy strains. Bartfai et al., (2003) genetically analyzed common carps from two farms by RAPD and microsatellite techniques. Ten RAPD primers for 80 individuals and four microsatellite primers for 196 fish were used for genotyping. Each method showed significant differences for two populations. Their heterozygosity value and allele frequency came out the same, but they did not show any groups on dendogram. Barman et al. (2003) used RAPD to distinguish four Indian carps. Khoo et al. (2003) crossbred wild and tuxedo strains and obtained F_1 and F_2 progenies. Then, they found low polymorphism within population, high polymorphism between two populations. Leuzzi et al. (2004) showed that *Astyanax altiparanae* species living in the Brazilian Paranapanema River had different variations in low stream by RAPD.

MATERIALS and METHODS

Fish

Eight strains of guppy (German, Fenerbahçe, blue, cobra, king, yellow pearl, flamingo, black) were used in this study. They were brought from an aquarium fish farm.

Sampling and DNA Isolation

The course of the experiment is demonstrated in Figure 1. Muscle samples were taken randomly from each fish (8 strains x 8 individuals). Fish were preserved in the 95% ethanol until beginning of DNA isolation fulfilled by Cordes et al. (2001).

RAPD-PCR

DNA was amplified by the PCR technique, which is used by Shikano and Taniguchi (2002). Nine random primers having 9-12 nucleotides were chosen to use (Table 1). Each PCR reaction included 50 ng

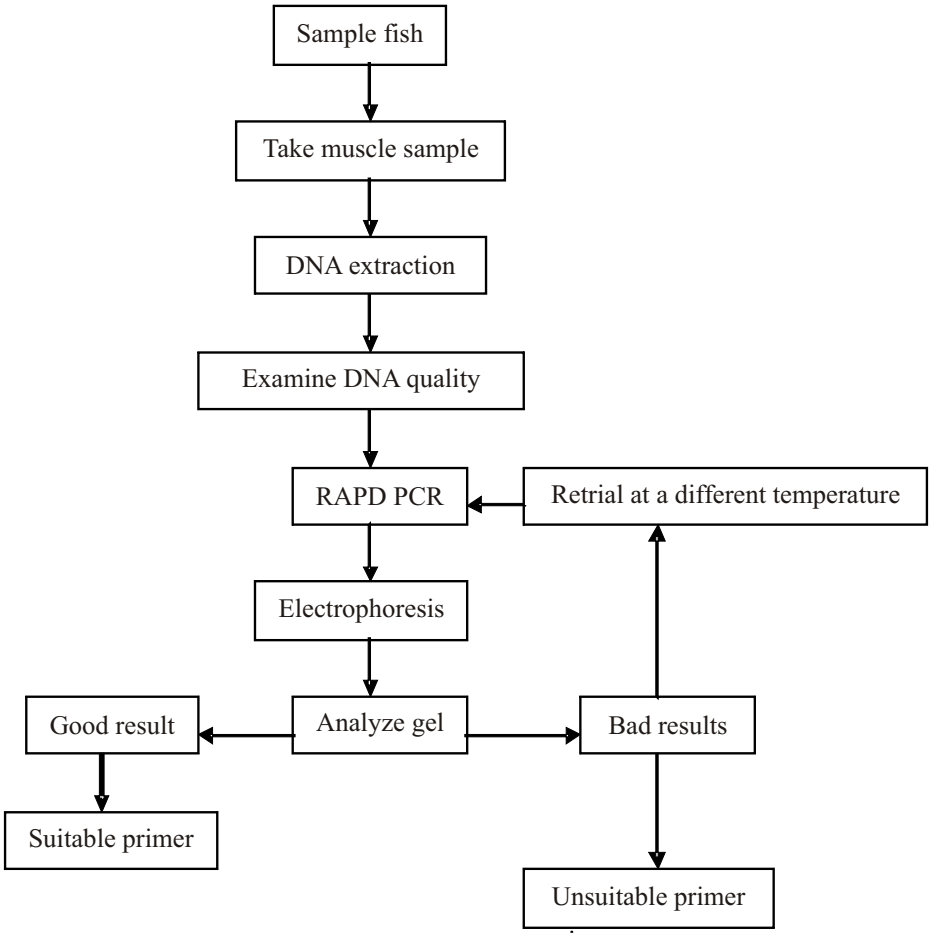

Figure 1. Scheme of genetic analysis

Table 1. Sequence of primer in this study

RAPD Primers	Sequence (5'-3')
A06	GCCAGCTGTACG
A08	GCCCCGTTAGCA
A10	ACTGGCCGAGGG
OPH15	AATGGCGCAG
OPJ04	CCGAACACGG
S102	AGGTGACCGT
S10D5	AGGTCACTGA
S11D6	TTGCGTCCA
S3D2	AATCGGGTCG

template DNA, 10 mM primer (MWG Biotech), 1.5 mM $MgCl_2$ (MBI Fermentas), 1.0 mM $dNTP_s$ (MBI Fermentas), 1 U Taq DNA polymerase (MBI Fermentas) and 2 l of 10x Taq buffer. Reaction volume was 20 l and the reactions were realized in a thermal cycler (Appligene Oncor Crocodile III). PCR cycle program followed such steps: heat-inactivation (94 C, 1 min), 40 cycles of denaturation (93 C, 1 min), annealing (30 C, 2 min), extension (72 C, 2 min) and final extension (72 C, 5 min). The PCR product was electrophoresed on 1.5% agarose gel. Gel was stained by ethidium bromide (10mg/ml) to see bands occurring.

Analysis

Bands of 64 individuals were analyzed to assess genetic variations between individuals and strains. According to bands of each sample and each primer, binomial matrixes were formed. After which genetic similarity (GS) was calculated (Nei and Li, 1979).

$$(GS)_{AB} = 2N_{AB} / (N_A + N_B)$$

$(GS)_{AB}$ = Genetic similarity between A and B

N_{AB} = the number of bands shared by A and B

N_A = the total number of bands in A

N_B = the total number of bands in B

GS ranges between 0 and 1. If GS is equal to 1, all bands are the same in individuals or strains. That is, there is no variation for this primer among them. If GS is equal to zero, individuals are different for that primer. Then genetic difference was calculated by following formula.

$$P = 1-(GS)_{AB}$$

Binomial data were statistically analyzed by JMP (SAS Institute, 1994) for Hierarchical Cluster Analysis.

RESULTS

Seven of nine primers were suitable for studying guppy DNA variations (Table 1). In RAPD analysis, 113 bands were obtained. The number of bands ranged between 1 and 7 for each strain and primer. Molecular sizes of bands were 25-615 bp within strains.

The average of genetic similarity was 0.832 in German, 0.919 in Fenerbahçe, 0.746 in blue, 0.785 in cobra, 0.670 in king, 0.800 in yellow pearl, 0.801 in flamingo, 0.948 in black. Within strains, the average of genetic difference was 0.168 in German, 0.081 in Fenerbahçe, 0.254 in blue, 0.215 in cobra, 0.330 in king, 0.200 in yellow pearl, 0.199 in flamingo, 0.052 in black.

Average genetic similarity and difference values between varieties are given at Table 2. The highest genetic similarity was 0.871 between German and Fenerbahçe, second yellow pearl and Fenerbahçe, third yellow pearl and German. The highest genetic variation was 0.604 between king and flamingo strains, second black and flamingo, and third cobra and flamingo. In flamingo genetic similarity was generally lower than all of the other strains.

Table 2. Average genetic similarity (S) and difference (D) between varieties

	GER	FB	BLU	COB	KIN	YP	FL
FB	S 0,871 D 0,129						
BLU	S 0,696 D 0,304	S 0,711 D 0,289 0.362					
COB	S 0,780 D 0,220	S 0,796 D 0,204	S 0,636 D 0,364				
KIN	S 0,718 D 0,282	S 0,701 D 0,299	S 0,574 D 0,426	S 0,687 D 0,313			
YP	S 0,803 D 0,197	S 0,826 D 0,174	S 0,667 D 0,333	S 0,783 D 0,217	S 0,701 D 0,299		
FL	S 0,555 D 0,445	S 0,501 D 0,499	S 0,471 D 0,529	S 0,459 D 0,541	S 0,396 D 0,604	S 0,490 D 0,510	
BLA	S 0,593 D 0.407	S 0,613 D 0,387	S 0,479 D 0,521	S 0,534 D 0,466	S 0,463 D 0,537	S 0,552 D 0,448	S 0,426 D 0.574

*GER: German, FB: Fenerbahçe, BLU: blue, COB: cobra, KIN: king, YP: yellow pearl, FL: flamingo, BLA: black

DISCUSSION

In this study, it is investigated if RAPD technique is a successful method to define genetic variations intrastrains and interstrains of guppy.

Variations of eight guppy strains were figured out. Genetic similarity in German, fenerbahce, blue, cobra, king, yellow pearl, flamingo, black were 0.832, 0.919, 0.746, 0.735, 0.670, 0.800, 0.801, 0.948, respectively. Intrastrain similarities were quite high because of inbreeding. Interstrain variations were found between 0,871 and 0,604. The highest genetic difference was between king and flamingo (0.604).

Hierarchical Cluster Analysis showed that only blue and black made two groups in themselves. Rest of them was grouped in a complex way.

Aquaculture experiments on improvement began to increase because genetic markers of positive development on. For market fish, weight, growth speed and feed conversion ratio are used as selection characteristics. However, selection based on such phenotypic characters does not provide genetic improvement because genotype and environment affect phenotype. Success will be exact by mean of selection with genetic identification markers. Before beginning of improvement in aquaculture, present fish population should be defined. After selection of brood stocks, improvement program will be started. To pick out brook stock variation among individuals is calculated. And then widely variable and scattered populations are preferred.

Development of genetic markers based on DNA revolution of all animal genetics. In theory, it is possible to determine genetic variation on genome. Allozyme, mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), RAPD, amplified fragment length polymorphism (AFLP), microsatellite and single nucleotide polymorphism (SNP) are frequent marker techniques. DNA markers increase development on determination of genetic variation and parents, definition of genetic map of cultured species. In addition, it is targeted to define the genes controlling quantitative trait loci (QTL) by using genetic markers.

Studies started first using allozyme technique on molecular genetic methods since 1970. Recently Human Genome Project whose research still continues and intensity of other researches on molecular genetics causes for occurrence of a new subject called *genomics* (works that is to determine DNA sequence and genes function for an organism).

RAPD, one of the DNA marker techniques was first developed in 1990 (Williams et al., 1990). RAPD technique is often used for examine of species genetics because of usage of random primers in PCR. For using RAPD, it is not necessary to know the genomic sequence of the organism. It makes this technique useful. It is possible to order any sequence of primers. In this doctorate study, used primers were chosen form previous studies (Shikano and Taniguchi, 2002; Foo et al., 1995, Khoo et al., 2003). If primer recognize a complementary sequence on genomic DNA, copy of this region is formed millions times and band consists. Otherwise, no bands occur. In this study, agarose gel electrophoresis was used due to its easiness and common usage. RAPD shows dominant inheritance and examines many loci at the same time and determines not more than two alleles. It is impossible

to take detail on individual polymorphisms.

There are many studies done on aquaculture by RAPD: determination of hybrids brown trout, *Salmo trutta* and Atlantic salmon, *Salmo salar* (Elo et al., 1997); examine of population structure of black tiger shrimps, identification of fish and mollusc species (Crossland et al., 1993; Hirschfeld et al., 1999; Klinbunga et al., 2000); determination of guppy species and its varieties and linkage map (Khoo et al., 2002). RAPD technique was tested on some ornamental fish (Goldberg et al., 2000 and Degani et al., 2000; Degani, 2002). Shikano and Taniguchi (2002) used guppy fish as a model for determinations of heterosis in F_1 offsprings by RAPD and microsatellite. In Turkey, Bilgen et al. (2002) investigated brook stocks of two hatcheries for sea bream, *Sparus auratus* in the Aegean region. Genetic variations were 0.380 in cultured fish and 0.550 in natural populations. Genetic variations among populations of three rainbow trout hatcheries were examined (Akhan, 2003). Genetic similarities were 0.428 for Fethiye, 0.310 for Çameli and 0.348 for Gölhisar. After inbreeding for many years, similarity went low because of aneuploidy.

In this study, genetic variations of interspecies ranged 0.074-0.320. Those of intraspecies were between 0.052-0.330. Genetic similarity of Mediterranean Sea bass within eight populations was lower than that of interpopulations (Caccone et al., 1997). Callejas and Ochando (1998) identified three barbus species with 7 RAPD primers. Genetic similarity was 41.23% between Barbus bocagei and B.graellsii, 39.81% between B.sclateri and B.bocagei, 38.27% between B.sclateri and B.graellsii. Genetic similarity of this study was the highest (87.1%) between German and Fenerbahce. It was the lowest (39.6%) between King and Flamingo.

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