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Use of Randomly Amplified Polymorphic DNA (RAPD) Analysis to Detect Genetic Variation in Sea Bass (*Dicentrarchus labrax*)

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Abstract: Genetic relatedness was estimated among five populations of the European sea bass (*Dicentrachus labrax* L.) using 9 RAPD (Randomly Amplified Polymorphic DNA) primers. Samples were collected from Egyptian coast Mediterranean (Al Borge, Meadea and Rashid) and other two populations were from Manzalla lake and Bardawil lagoon. These primers produced 94 bands that could be scored with high confidence. On average, each primer gave rise to 6-16 bands and a majority of the bands was polymorphic. The percentage of polymorphic bands in Al-Borge (45%) and Meadea (44%) populations was low compared to Rashid (55%) and Manzalla (52%) populations. RAPD analysis showed that the Bardawil population had higher genetic polymorphism (64%) than the other populations. The phylogenetic tree constructed by unweighted pair-group method of analysis (UPGMA) shows the Al-Borge and Manzalla populations and Meadea and Rashid populations, respectively, seems to be approximately as closely linked to each other from the dendrogram. The Bardawill population is more related to the Rashid and Manzalla populations. High levels of genetic variation and population differentiation indicated dynamic evolution in these populations as revealed by variation at RAPD loci.

Key words: European sea bass, (*Dicentrachus labrax* L.), genetic variability, genetic distance, RAPD

INTRODUCTION

The European sea bass (*Dicentrachus labrax* L.), has a widespread distribution and ranges from Turkish coasts of the Black Sea, Sea of Marmara, Aegean Sea, Mediterranean and Atlantic coasts from Spain, Portugal, Morocco coast and North Sea, Baltic Sea and North America (Smith *et al*., 1990; Nelson, 1994). The reproduction of sea bass occurs in marine coastal areas. Eggs and larvae drift towards estuaries and lagoons by passive movements, although larvae actively search for low salinity water. Young bass stay in these protected coastal areas for approximately 2 years. Juvenile sea bass engage in sporadic and occasional migrations which are often geographically restricted (Pickett and Pawson, 1994).

Along the North coastal Egyptian waters, lagoons and mouths of Nile River, sea bass is a commercially important resource. The increasing fishing and aquaculture effort exerted upon sea bass along the Egyptian coastal waters and the differences in exploitation patterns between coastal areas, demand knowledge of the stock structure and the degree of mixing among populations to adequately manage this important species. In order to manage a fishery effectively, it is important to know the identity of stock structure of the species, as each stock must be managed separately to optimise their yield (Grimes *et al*., 1987). Disregard of stock structure and ineffective fishery management can lead to dramatic changes in the biological attributes and the productivity of a species (Altukhov, 1981; Smith *et al*., 1991). The European sea bass, *Dicentrarchus labrax*, is a promising species for Egyptian fish farming, owing to its high economic value, fast growth and need for market diversification. Natural

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populations of the European sea bass (*Dicentrachus labrax* L.) have been the subject of many studies using several types of genetic markers, like an Allozymes (Allegrucci *et al*., 1997; Castilho and Mc Andrew, 1998), Microsatellites (Garcia de Leon *et al*., 1995; Castilho and Mc Andrew, 1998) and mitochondrial DNA (Cesaroni *et al*., 1997). However, little of genetic variance information exists so far on the genetic variability of the sea bass populations in Egypt. Determining the genetic variation between and within sea bass collections is the first step toward developing, improving and avoiding genetic erosion for Egyptian sea bass.

Random Amplified Polymorphic DNA (RAPD) is a useful methodology to assess genetic variations in fish populations. The methodology can detect high levels of DNA polymorphisms and can produce fine genetic markers (Williams *et al*., 1990; Welsh and McClelland, 1990). RAPD technology is a reliable method for characterizing variation within and among species and populations (Excoffier *et al*., 1992). RAPD polymerase chain reaction has been used for monitoring genetic changes in the acclimation of the European sea bass to freshwater (Allegrucci *et al*., 1995). Furthermore, genetic differentiation within and among European sea bass (*Dicentrachus labrax* L.) from different locations using RAPD showed high levels of polymorphism (Caccone *et al*., 1997). The objective of this work was to study the genetic variation by using RAPD in different populations of sea bass.

MATERIAL AND METHODS

Sample Collection and DNA Extraction

Fish samples were collected from five different sites in Egypt (Fig. 1). Three sites were from Egyptian coast Mediterranean (Al Borge, Meadea and Rashid) and other two populations were from Manzalla Lake and Bardawil lagoon. These sites have diverse environmental conditions in terms of average water salinity and temperature. Fin clips tissue was collected from 250 *Dicentrarchus labrax*, L. individual fish, freshly obtained from commercial fishermen and immediately preserved in 95% ethanol. Whole genomic DNA was extracted from individuals according to the method of Taggart *et al*. (1992). The DNA quality was checked by electrophoresis in a 1% agarose gel and the concentration was estimated in relation to the concentration of co-migrating λ-phage DNA and by repeated measurements with spectrophotometer at 260 nm.

RAPD Reactions

A total of 250 individual fish representing 5 populations (fifty individuals per population) were assayed using nine primers (OPA-05, OPA-09, OPA-17, OPB-10, OPB-17, OPC-07, OPC-11, OPC-13 and OPC-15). All primers were from Operon Technologies (CA, USA) (Table 1). The reproducibility of the banding patterns of each primer was tested with respect to the amplification conditions, concentration of the primer relative to the template DNA and magnesium chloride concentration. Once optimal conditions had been determined for each specific primer, the conditions were strictly followed. DNA amplification was performed in a Perkin Elmer Thermal Cycler with one cycle at 94°C for 5 min, 36°C for 2 min and 72°C for 2 min, followed by 35 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final cycle at 72°C for 7 min. Reactions were carried out in 25 μL volumes containing 1X DNA polymerase buffer [50 mm Tris-HCl (pH 8.5), 2.0 mm $MgCl_2$, 50 mm KCl, 0.1% Triton X-100], 0.2 mm of each dNTP, 0.4 μm of primer, 1 U AmpliTaq DNA polymerase and 20 ng of DNA. The reaction mix was overlaid with a drop of mineral oil to avoid evaporation during the cycling. Amplification products were electrophoresed in 1.5% agarose gels in TBE (0.5×) buffer and detected after ethidium bromide staining according to Sambrook *et al*. (1989). Amplifications were performed at least twice and only reproducible products were taken into account for further data analysis.

Table 1: Sequences and operon codes of the random primers used to detect of variation in sea bass

Primer codes	Sequences (5' to 3')
OPA-05	AGGGGTCTTG
OPA-09	GGGTAACGCC
OPA-17	GACCGCTTGT
OPB-10	GGACTGGAGT
OPB-17	AGGGAACGAG
OPC-07	GTCCCGACGA
OPC-11	AAAGCTGCGG
OPC-13	AAGCCTCGTC
OPC-15	CTCACCGTCC

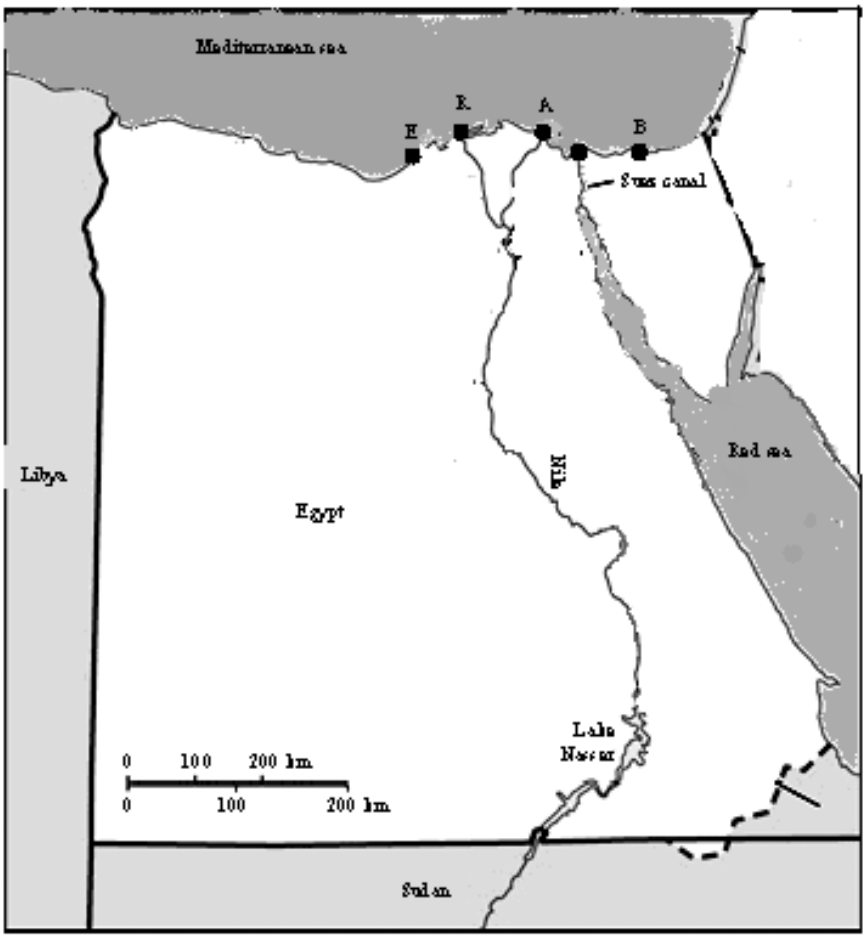

Fig. 1: Map of Egypt, showing the sampling sites. A: Al-Borge, E: Meadea, R: Rashid, M: Manzalla and B: Bardawil

Data Scoring and Analysis

Each amplified DNA fragment was considered as an independent character (locus) and scored as present (1) or absent (0). Since RAPD markers are dominant, a locus was considered to be polymorphic if the presence and absence of the bands were observed in various individuals and monomorphic if the bands were present in all individuals. RAPD data was used to determine gene diversity, number of polymorphic loci and genetic distance and to construct an unweighted pair group

method of arithmetic mean (UPGMA) dendrogram among populations using DISPAN (Genetic Distance and Phylogenetic Analysis) (Nei *et al*., 1983) and GenAIEx6 (Genetic Analysis in Excel) software.

RESULTS

Genetic Variability

A total of 9 primers were used to investigate five populations. Each of the random primers produced polymorphic banding patterns in all of the populations examined. The nine primers produced a total of 94 easily scorable RAPD bands in replicate amplifications that were used for assessing genetic variation within and among the five populations. These bands ranged in molecular size from approximately 300 to 3000 bp. Out of the 94 amplification products scored, 87 bands (93%) were found to be polymorphic. The average number of scoreable bands per primer was 10.44 (ranging from 6 (OPC13) to 16 (OPB17) bands) and the average number of polymorphic bands (PPB) was 9.77 (ranging from 5 (OPC13) to 16 (OPB17) bands) (Table 2). The average of number of polymorphic bands detected was lower for Meadea population 4.66 bands while the Bardawil population was 6.77 bands (Table 2). No characteristic and/or diagnostic bands were found for any populations. The average heterozygosity within populations was the highest (0.363) for Bardawil population compared with other populations (Table 2).

Genetic Distance

The genetic distances were calculated according to Nei *et al*. (1983). Genetic distance among different populations ranged from 0.110 between Manzalla population and Al-Borge population to 0.218 between Rashid population and Al-Borge population (Table 3). The distance matrix based on RAPD data not sets is graphically represented as a dendrogram using the UPGMA method shown in Fig. 2. The dendrogram linked Manzalla and Al-Borge populations separated from Meadea and Rashid populations with the Bardawill population is branching away from the rest of the populations and seemingly is more distant related to the other population of sea bass (Fig. 2).

Table 2: Polymorphic amplified bands and mean heterozygosity detected with nine primers for five populations of *D. labrax* (percentages of polymorphic band, PPB)

		No. of polymorphic bands (PPB)					Total No. of polymorphic bands (PPB)
Primer	No. of bands amplified	Al Borge	Meadea	Rashid	Manzalla	Bardawil	
OPA-05	10	5 (50%)	8 (80%)	5 (50%)	6 (60%)	6 (60%)	8
OPA-09	13	4 (30%)	7 (53%)	4 (30%)	7 (53%)	9 (69%)	12
OPA-17	14	7 (50%)	6 (42%)	6 (42%)	7 (50%)	9 (64%)	14
OPB-10	7	3 (42%)	4 (57%)	5 (71%)	6 (85%)	5 (71%)	6
OPB-17	16	8 (50%)	3 (18%)	8 (50%)	7 (43%)	9 (56%)	16
OPC-07	11	5 (45%)	2 (18%)	7 (63%)	7 (63%)	10 (90%)	11
OPC-11	9	4 (44%)	4 (44%)	7 (77%)	4 (44%)	7 (77%)	9
OPC-13	6	2 (33%)	4 (66%)	3 (50%)	1 (16%)	2 (33%)	5
OPC-15	8	5 (62%)	4 (50%)	7 (87%)	4 (50%)	4 (50%)	7
Average	10.44	4.77 (45%)	4.66 (44%)	5.77 (55%)	5.44 (52%)	6.77 (64%)	9.77 (93%)
Average heterozygosity		0.226	0.250	0.283	0.305	0.363	

Table 3: The genetic dissimilarity matrix among the five populations of sea bass based on RAPD data

Populations	Al-Borge	Meadea	Rashid	Manzalla	Bardawil
Al-Borge	0.000				
Meadea	0.168	0.000			
Rashid	0.218	0.111	0.000		
Manzalla	0.110	0.202	0.202	0.000	
Bardawil	0.204	0.160	0.129	0.183	0.000

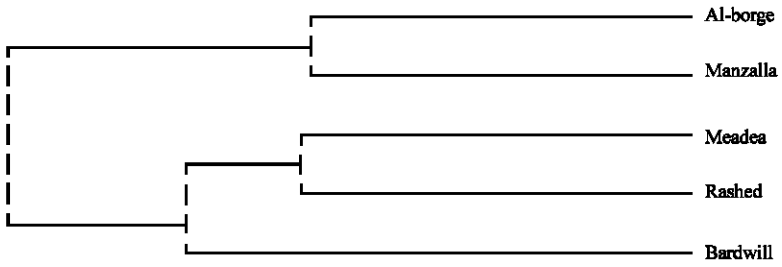

Fig. 2: Unweighted pair group method of arithmetic mean dendrogram based on Nei *et al*. (1983) genetic distance, summarizing data on differentiation between Tenualosa ilisha populations according to random amplified polymorphic DNA analysis

DISCUSSION

The present study shows genetic variation within and between the European sea bass (*Dicentrachus labrax* L.) populations of five locations of Egypt, indicating the presence of separate stocks of sea bass that may be due to the sea or lagoon ecology, spawning grounds, nursery grounds of the juveniles and seasonal migration (Brahmane *et al*., 2006).

The sea bass (*Dicentrarchus labrax* L.) is one of the most economically important cultured marine species in Europe. However, 20 years of large-scale production of sea bass has not yet generated a single domesticated stock (Chistiakov *et al*., 2004). Understanding of genetic diversity across the species range could be of great importance for the future development of aquacultural strains, for the protection of small-endangered populations and for biogeographical inferences (Hassanien *et al*., 2004). Genetic variation within and among populations is essential to their ability to survive and successfully respond to environmental changes (Ryman *et al*., 1995). The result of the present study has demonstrated that the RAPD technique could be applied for measuring the degree of variability within and between sea bass populations (Allegrucci *et al*., 1994; Caccone *et al*., 1997). RAPD can be an efficient tool to differentiate geographically and genetically isolated populations and has been used to verify the existence of locally adapted populations within a species that may have arisen either through genetic selection under different environmental conditions or as a result of genetic drift (Fuchs *et al*., 1998).

The UPGMA cluster analysis divided the genotypes studied into two main groups. The first group is Manzalla and Al-Borge populations and the second group is Meadea and Rashid populations. The Bardawil population is branching away from the rest of the populations and seemingly is more distant related to the other population of sea bass. Population genetic differentiation can be driven by ecological, evolutionary and historical factors. In *Barbus neumayeri*, genetic differentiation among sampling sites that presented different oxygen rates could represent the effects of selective pressure (Chapman *et al*., 1999). The well-developed homing instinct of salmonid fish seems to be a decisive factor leading to strong population subdivisions (Ryman, 1983). An evolutionary unit can be identified for each tributary, with particular genetic traits possibly related to local adaptation and/or to inbreeding. In *Oncorhynchus nerka*, genetic differences were found between two populations inhabiting regions with distinct environmental conditions (Hendry *et al*., 2000). Furthermore, some river or lake systems contain metapopulations composed of distinct breeding units (Carvalho, 1993; Hansen and Loeschcke, 1994).

RAPD analysis has some limitations that must be considered. It shows dominant inheritance and marker/marker homozygotes cannot be distinguished from marker/null heterozygotes. In addition, it is unable to assign bands to specific loci unless a previous pedigree analysis is performed. In applying

this method, it is assumed that populations are under the Hardy-Weinberg equilibrium that polymorphic bands segregate in the Mendelian way and that marker alleles from different loci do not comigrate to the same position in the gel (D'Amato and Corach, 1996). However, before making the final decision to select the sea bass populations to be conserved and use for selection breeding program, I recommend further analysis using co-dominant molecular markers like mitochondrial and microsatellite markers, will further enhance our understanding of the genetic stock structure of sea bass in Egypt.

The genetic distance shows a distance range from 0.110 to 0.218 (Table 3). Thus, the accessions tested in this study are highly divergent at the DNA level. The smallest distance value of 0.110 was observed between Al-Borge and Manzalla which seem to be nearly similar. The maximum distance value of 0.218, suggesting great dissimilarities, was observed between Al-Borge and Rashid. The population genetic variance of sea bass suggests that there are significant population subdivisions in both Atlantic and Mediterranean parts of its range (Bahri-Sfar *et al*., 2000; Castilho and McAndrew, 1998; García de León *et al*., 1997; Naciri *et al*., 1999). Microsatellites markers reveal a bigger internal variability for sea bass inside the East Mediterranean group than in western populations (Bahri-Sfar *et al*., 2000). Microsatellites are suitable tools to assess the geographical structure of sea bass populations but they do not reveal any differences in terms of ecological partitioning, unlike allozymes (Lemaire *et al*., 2000). Based on allozymes, it is also possible to discriminate between fish living in coastal (offshore) and lagoon (inshore) environments, possibly providing evidence for selection (Allegrucci *et al*., 1995, 1997). These authors showed a clear genetic homogeneity among sea bass populations living in various Mediterranean coastal lagoons, independently of their geographic distance. Such high homogeneity could be explained by migration of particular genotypes into coastal lagoons.

The data generated in this study provide useful information on of the genetic variation among different population of sea bass. This information can be applied to design suitable management guidelines for this stock or others from the same or related species.

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