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Molecular Characterization of the Whiting (*Merlangius merlangus euxinus* Nordmann, 1840) in Turkish Black Sea Coast by Rapd Analysis

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Abstract: The Random Amplified Polymorphic DNA (RAPD) technique offers an useful tool to investigate DNA polymorphisms. It can be used to distinguish different populations belong to one species. These markers also represent an efficient and inexpensive way to generate molecular data and thus, they have been used successfully in various taxonomic and phylogenetic studies. Information on the genetic structure of native fish populations is essential for studying molecular systematics and optimising fisheries management. RAPD assay was evaluated for studying genetic relationships and diversed in eight populations of whiting (Family: Gadidae). We used RAPD to determine the genetic characterization and the stock differentiation of whiting, *Merlangius merlangus euxinus*, eigth populations in the Black Sea coast of Turkey by using eleven arbitrary primers. The genetic relationship among the determined eight stations was estimated according to Jaccard similarity index and cluster analysis. Jaccard similarity coefficient values ranged from 0.676 to 0.836. The lowest similarity (0.676) was found between Karasu ve Rize and the highest similarity (0.836) was between Kıyıköy ve Zonguldak. In consequence of cluster analysis, two stations were classified in the first branch of the derived dendogram while the others were classified in the second branch. The average similarity between the two branches was 0.720. Among the primers tested, OPAB-01, 08, 14, 17, OPA-08, 12, 19, OPB-08 and OPC-11 showed polymorphic bands. Amplified fragments ranged from 218 to 2196 base pairs and the numbers of bands for each primer varied from 2 to 9.

Key words: Whiting, *Merlangius merlangus euxinus*, RAPD, genetic similarity, Black sea

INTRODUCTION

Whiting is a common gadoid fish in the Northeastern Atlantic and the Mediterranean. Whiting, *Merlangius merlangus euxinus* (Nordmann, 1840) (Teleostei, Gadidae), is one of the most abundant and economically important fish species in the Black Sea. It has two subspecies of whiting (Two subspecies, *M. m. euxinus* and *M. m. merlangus*, are distributed) in the North-eastern (Northeastern), Atlantic (Ocean) and Mediterranean. These subspecies are identified by barbel on chin and pectoral fin lenght. *Merlangus merlangus euxinus* has a conspicuous barbel on chin. It is common along the European coasts of the mediterranean, in the Black Sea and Azov Sea (Slastenenko, 1956; Fisher, 1973; Whitehead *et al*., 1986).

RAPD technique consists in the amplification, by Polymerase Chain Reaction (PCR), of random segments of genomic DNA using a single short primer of arbitrary sequence, thus, one can expect to scan the genome more randomly than using conventional techniques. The examination investigation of genomic variation without previous sequence information shows that the relatively low cost of the technique and requirement of only nanograms of template DNA provide advantages in the use of RADP in population and other genetic studies (Williams *et al*., 1990). Thus, the RAPD-PCR method has been used successfully to detect genetic variation within and among related species and populations of different organisms, including fishes (Dinesh *et al*., 1993; Bardakci and Skibinski, 1994; Bielawski and Pumo, 1997; Borowsky *et al*., 1995; Chen and Liebenguth, 1995; Foo *et al*., 1995; Sultmann and Mayer, 1995). RAPD analysis also has been used to evaluate genetic diversity for species and subspecies identification in guppy (Dinesh *et al*., 1993), Tilapia (Bardakci and Skibinski, 1994; Dinesh *et al*., 1996), brown trout and Atlantic salmon (Elo *et al*., 1997) largemouth bass (Williams *et al*., 1998) and Ictalurid catfishes (Liu *et al*., 1998). So far, it has not been used to study in whiting populations. The specific objectives of the present study were to evaluate using RAPD assay as a source of genetic markers to estimate genetic variation among these eight whiting populations.

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Previous studies were related with the distribution, abundance and stock assessment of whiting, *M. m. euxinus*, in the Turkish Black Sea coast by Akşiray (1954), Bingel *et al.* (1991, 1993), Kutaygil and Bilecik (1979), Düzgüneş and Karaçam (1990). There is no data on differentiation of whiting stocks along the Turkish Black Sea coast. Ismen (2001) studied stock differentation of whiting in Turkish Black Sea Coast by applying the generalised distance of Mahalanobis according to both morphologic and meristic data. Insufficient differences ($p>0.01$) in general phenotypic and genotypic characteristics implied the existence of a single unit stock.

The aim of the present study is to determine genetic variations among whiting populations in the Turkish Black Sea coast The phylogenetic relationships among eigth native populations were studied by using RAPD markers. Cluster analysis of data from twenty random primers placed the eigth populations. A dendogram was generated using the Unweighted Pair-group Method with Arithmetical Averages (UPGMA) as described by Sneath and Sokal (1973).

MATERIALS AND METHODS

Sample collection: A total of 270 whiting, *M. m. euxinus* individuals were collected from eight various localities in the Turkish Coast of the Black Sea during from April to May in 2002 (Fig. 1). Each specimen was labelled with a tag inserted into the operculum then and kept at -70°C until DNA extractions.

DNA extraction: Genomic DNA was isolated from white muscle tissue. Approximately 20 mg of tissue was removed using sterile scalpel blades and forceps. DNA was extracted from frozen white muscle tissue by the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, USA). Proteinase K was used during extractions to promote cell lysis and protein digestion. In order to remove RNA, the resuspended DNA was treated with RNase A (1 μg per 100 μL total volume) and incubated at 37°C for 30 min. Extracted DNA was resuspended in DNA rehydration solution. The concentration of extracted DNA was determined by using Gene Quant RNA/DNA Spectrophotometer (Pharmacia Biotech, England) and stored at -20°C.

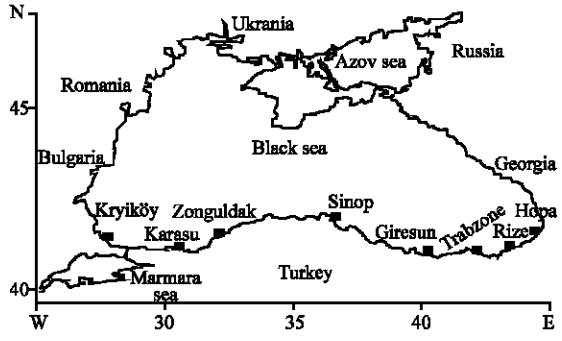

Fig. 1: The sampling stations along Turkish Black Sea coast

RAPD analysis: In RAPD analysis, different PCR amplification conditions (concentration of template DNA, dNTP, Mg and primer, temperature and time of denaturation, annealing and extension) were performed for PCR optimisation and the best reaction conditions were selected.

Two hundred and seventy specimens were screened with twenty decamer primers. Twenty primers (Operon RAPD 10-mer Kits, Set AB, B ve C, OPERON Tech. Inc., Alameda, CA, USA) were arbitrary chosen for preliminary screening. Eleven primers that gave reproducible results in two independent DNA extractions were then chosen for further analysis. The experiments were carried out with varying concentrations of MgCl2, dNTPs, and DNA template in order to optimize the PCR conditions. The PCR reactions were performed in a final volume of 25 μL containing 2.5 mm 10× Reaction buffer, 2.5 mm MgCl2, 200 μm each of dNTPs, 0.2 μL of each arbitrary primer, 80 ng template DNA, and 1 unit of Taq DNA polymerase overlayed with approximately 25 μL of mineral oil to prevent evaporation. Amplification reactions were duplicated to ensure reproproducibility. PCR was carried out with a Hybaid PCR Sprint (Hybaid Ltd, UK) Thermal Cycler programmed for 1 cycle of 3 min at 94°C followed by 45 cycle of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. The last cycle was followed by a final incubation at 72°C for 6 min. Twenty random primers were arbitrarily chosen for preliminary screening. These primers that gave reproducible results were then chosen for further analysis. The amplification products were analyzed by electrophoresis on 1.4% agarose gels at 94 volts for 45 min. pUC 18 plasmid DNA digested with Hinf I was used to determine the molecular size of bands. The gels were stained with ethidium bromide and exposed under UV lights. The fragment patterns were photographed for further analysis.

Data analysis: Amplified bands were visually scored as present or absent. A similarity matrix was generated by using the Sneath and Sokal (1973) similarity index based on the proportion of shared amplification fragments between two genotypes. A dendrogram was constructed based on the similarity indices data by applying

Unweighted Pair Group Method with Arithmetic Averages (UPGMA) cluster analysis using the NTSYS-pc computer program (Rohlf, 1990).

RESULTS

In present study, highly pure DNA was isolated from white muscle tissues of the specimens from eigth different populations by Promega Genomic DNA Isolation Kit and a gene pool of 10 individuals representing each station was constituted. DNA concentrations were determined using the spectrophotometeric method. The DNA concentrations ranged from 165 to 436 μg DNA per g of white muscle.

While selecting the primers, 20 decamer primers which were designed by Operon Tech. Inc. suitable for RAPD studies were experinced under PCR amplication conditions and according to the results, eleven of them were selected and used in the present study. Numbers of bands derived by these primers in eigth different stations are given in Table 1. PCR products were analyzed by electrophoresis and the gels were photographed for further analysis (Fig. 2).

A total of 270 whiting specimens were investigated using twenty arbitrarily selected primers. Totaly, 517 bands were generated by PCR reactions. Each RAPD analysis was repeated three times and the same results were derived. The total numbers of bands amplified by these selected primers vary from 16-65 (Table 1). Polymorphic RAPD fragments range from 0.21-2.19 kb pairs (kb) in eigth whiting population. The average number of polymorphic bands varied from 1 to 8. In particular, primers OPA-08 and OPC-11 produced highest number of fragments among the primers used, with an average of 6-9. OPA-08 primer generated 65 bands in all stations. On the other hand, primer OPA-05 produced the lowest number of fragments with an average of 2. OPA-05 primer generated 16 bands in all stations. OPA 05 and OPA 10 generated the same bands in all samples (Table 1). According to these bands all regions were seems to be identical while other primers very little differences were observed by other primers.

It was generated a similarity matrix by using the Sneath and Sokal (1973) similarity index based on the proportion of shared amplification fragments between two genotypes (Table 2).

In this study, UPGMA cluster analysis of the similarity matrix separated whiting populations into two groups. The first group contains Karasu and Sinop while the second group contains Kıyıköy, Zonguldak, Giresun, Hopa, Trabzon and Rize. In the first group, the dendrogram clearly showed that Karasu and Sinop were closely related, with a similarity index of 0.826 (Fig. 3). The second group has two sub-groups containing two branches, Kıyıköy/Zonguldak/Giresun and Trabzon/Hopa /Rize combinations. Both of the combinations have two branches. In the first branch of first group, while Kıyıköy and Zonguldak connected with a similarity index of 0.836, Giresun connected to this combination with a similarity index of 0.757. In the first branch of first group, while Trabzon and Hopa connect with a similarity index of 0.820, Rize connected to this combination with a similarity index of 0.779 (Fig. 3).

Table 1: DNA sequences of random decamer oligonucleotide primers used for DNA amplifications of *M. merlangus euxinus*

Desisnations	Base sequence (5'→3')	Approximate range of fragment size (kb)	Total No. of amplified products	No. of polymorphic bands	% of polymorphbands
OPAB 01	CCGTCGGTAG	0.2-1.366	45	2	4.4
OPAB 08	GTTACGGACC	0.2-2.164	21	1	4.7
OPAB 14	AAGTGCGACC	0.3-2.196	32	8	25
OPAB 17	TCGCATCCAG	0.2-1.502	49	2	4
OPA 05	AGGGGTCTTG	0.9-1.487	16	0	0
OPA 08	GTGACGTAGG	0.2-1.564	65	3	4.6
OPA 10	GTGATCGCAG	0.3-1.138	24	0	0
OPA 12	TCGGCGATAG	0.3-1.385	38	4	4.6
OPA 19	CAAACGTCGG	0.2-1.521	45	2	4.4
OPB 08	GTCCACACGG	0.3-1.273	47	2	4.2
OPC 11	AAAGCTGCGG	0.2-1.060	64	3	4.6

Table 2: Similarity indices among whiting populations based on RAPD analysis

Kıyıkoy	Karasu	Zonguldak	Sinop	Giresun	Trabzon	Rize	Hopa	
Kıyıkoy	1.000							
Karasu	0.740	1.000						
Zonguldak	0.836	0.735	1.000					
Sinop	0.698	0.826	0.688	1.000				
Giresun	0.791	0.757	0.723	0.681	1.000			
Trabzon	0.775	0.736	0.742	0.719	0.800	1.000		
Rize	0.788	0.676	0.694	0.700	0.693	0.758	1.000	
Hopa	0.755	0.765	0.714	0.764	0.757	0.820	0.800	1.000

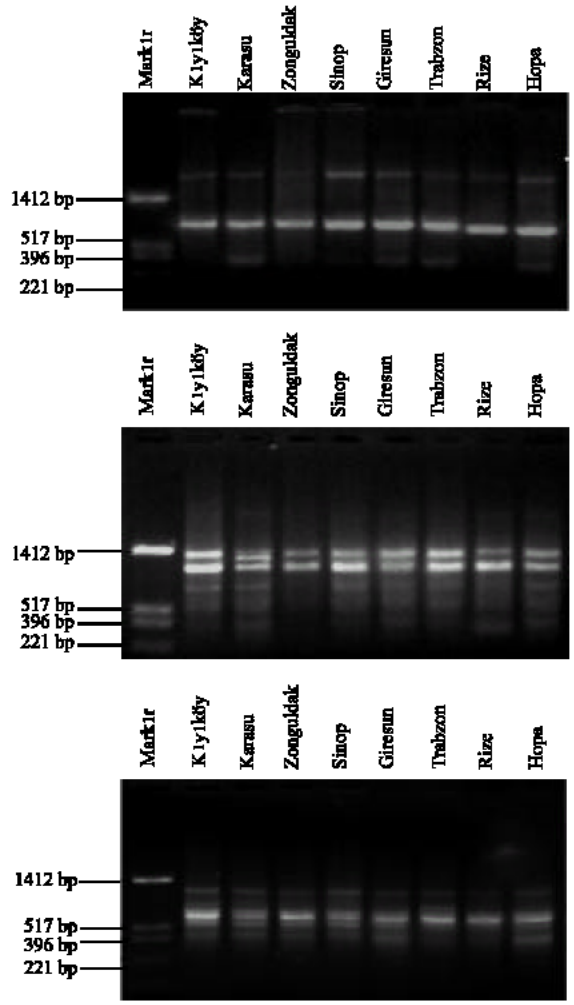

Fig. 2: RAPD profiles generated with OPB08, OPA12 and OPAB08 separated on 1.4% agarose gels, respectively. Line 1: pUC18 plasmid digested with Hinf I

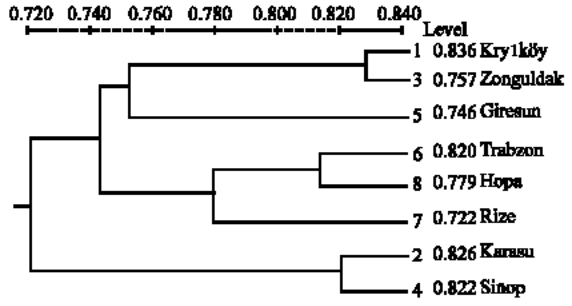

Fig. 3: UPGMA cluster analysis of RAPD data generated by eleven random primers for eight location of *M. merlangus euxinus*

DISCUSSION

RAPD could be an efficient tool to differentiate geographically and genetically isolated populations, and it 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).

Transportation and mixing processes can provide an exchange of genes between the fishes (fish) inhabiting the basin of the Black Sea despite the border of the eastern and western cyclonic gyres, which could be considered an oceanographic barrier for eggs and larvae of the eastern and western Black Sea whiting.

Eggs and larvae of whiting may be transported in as little as about 54 days along approximately 1400 km of Anatolia shoreline at a mean current speed of 30 cm s^{-1}. Hislop (1975) stated that an individual female spawns in batches and its spawning season lasts at least ten weeks (75 days). Russell (1976) stated that the incubation period of the eggs based on their temperature is generally 12-15 days and that 5.5 mm length is reached within 5 days after hatching, when the postlarval stage begins. During the time periods mentioned by Hislop (1975) and Russell (1976) eggs and larvae may be transported and mixed either completely or partly. These processes may allow the exchange of genetic characteristics between fish inhabiting the western and eastern basins of the Black Sea and, as a result, may sufficiently dilute any differences in general phenotypic and genotypic characteristics, so as to imply the existence of a single unit stock.

Whiting populations inhabit along different geographic regions of Turkish Black Sea coasts adapted to the ecologic conditions of the region; such as food, stream, temperature and salinity and because of this fact, genetic differences has occurred relatively.

Stock differentiation studies of whiting, *Merlangius merlangus euxinus*, from the Turkish Black Sea coast were carried out using morphometric and meristic characters and applying the generalised distance of Mahalanobis. The little difference between the general phenotypic and genotypic properties reveals only one stock in the Black Sea (Ismen, 2001).

Primer screening regions are very small in proporation to the genomic DNA. Probability to exclude gene regions coding the particular characteristics in amplified regions may cause incorrect results when evaluating genetic similarity findings. It is a particular point that the ecologic similarities in a population consist of individuals adapted to a specific geographical region should be parallel to genomic similarities.

The RAPD analysis has produced 0.720 similarity index between the two main groups. Giresun and Rize don't combine directly with any of the other regions but combine to other groups with secondary connections. The connection of Giresun and Rize with a low degree of similarity with 0.757 to Kıyıköy/Zonguldak and with 0.779 to Trabzon/Hopa combinations can be explained that these two groups have closer local habitats. The differences derived by RAPD analysis are not enough to distinguish the populations. And the little differences observed among populations may be the effects of important factors such as; temperature, stream, feeding conditions, amount of toxic materials in ecological environment of individual. The results confirm the results of stock discrimination studies (Ismen, 2001) done by using both morphologic and meristic characters. So we also suggest that there is only one whiting stock in the Black Sea as reported by Ismen (2001).

CONCLUSION

This is the first report on the use of DNA-based polymorphism assay that contributes assessing phylogenetic relationship among whiting populations in Turkish Black Sea Coast.

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