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Genetic Diversity of Gilthead Seabream (*Sparus aurata*) Broodstocks as Determined by RAPD-PCR

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Key words: RAPD-PCR, gilthead seabream, *Sparus aurata*, broodstock, genetic diversity

Abstract

The objective of this study was to determine the genetic diversity in gilthead seabream broodstocks from two hatcheries on the northern Aegean Coast of Turkey by RAPD-PCR. Forty primers were tested for each broodstock. Twenty-five produced scorable RAPD bands in stock from the Seferihisar hatchery and 28 in stock from the Aliaga hatchery. Nineteen revealed patterns with scorable amplified primers in both broodstocks. Depending on primer, the number of bands varied 3-16, ranging in size from 438 to 2520 base pairs (bp). The average genetic similarity within stocks was 0.466 for the Seferihisar stock and 0.617 for the Aliaga. The average genetic similarity between the two broodstocks was 0.420, lower than the values within the broodstocks, and the genetic distance between the two broodstocks was 0.245.

Introduction

The gilthead seabream (*Sparus aurata*) inhabits the Mediterranean and Atlantic coasts of Europe and is one of the most important cultured marine fish in the Mediterranean and Adriatic Seas. The commercial importance of gilthead seabream has generated intense interest in its molecular genetics. Funkenstein et al. (1990) first reported on mitochondrial DNA polymorphism of gilthead seabream broodstocks in Israel by using RFLP analysis of the whole mitochondrial DNA (mtDNA) molecule. Magoulas et al. (1995) reported on mtDNA polymorphism for Greece broodstocks. Palma et al. (2001) studied developmental stability and genetic heterozygosity in wild and cultured gilthead seabream stocks. Alarcon et al. (2004) compared cultivated and wild seabream stocks from the Atlantic and Mediterranean coasts using allozymes, microsatellite, and mtDNA markers. De Innocentiis et al. (2004) investigated the genetic variability of gilthead seabream populations from the Atlantic Ocean, Mediterranean Sea, and Adriatic Sea using microsatellite markers.

In Turkey, mariculture of seabream has

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continuously grown since it began in the late 1980s. The aim of the present study was to determine the genetic diversity within and between two Turkish gilthead seabream broodstocks using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR), a relatively simple, quick, and inexpensive technique that requires no target DNA sequence information.

Materials and Methods

Fish samples. Gilthead seabream (4 years old) were provided by two private hatcheries in Seferihisar and Aliaga, in Izmir province, western Turkey. The Seferihisar broodstock originated from Sigacik Bay and the Bostanli coast of Izmir Gulf (Fig. 1). The Aliaga broodstock originated from the Karatas lagoon in the Adana province of eastern Mediterranean. All broodstock were captured as juveniles and reared in cage farms until market size. Then they were transferred to hatcheries and used for seed production. Fifteen randomly chosen fish from each hatchery were used in this study.

DNA extraction. Blood samples (0.5-1 ml) were collected from the caudal vein using a 5-ml EDTA-coated syringe. Samples were put into separate 5-ml tubes containing K_3-EDTA. DNA was isolated from the samples according to Dunnington et al. (1990). The DNA pellet was dissolved in 100 µl of TE buffer (10mM Tris, 1mM EDTA, pH 8) and the concentration of each DNA sample was checked by spectrophotometer.

RAPD-PCR. Forty 10-mer random oligonucleotide primers (OPA and OPB, each series consisting of 20 primers; Operon Technologies, Alameda, CA, USA) were used to amplify the genomic DNA. Amplification reactions were carried out on a final volume of 15 µl containing 25 ng DNA, 100 µM each of

Fig. 1. Hatcheries and sampling regions. ● = hatcheries; ▲ = origins of Seferihisar broodstock: BS = Bostanli coast, SB = Sigacik Bay; ▼ origin of Aliaga broodstock: KL = Karatas lagoon.

dATP, dTTP, dCTP, and dGTP (Boehringer Mannheim, Germany), 15 ng of the primer, 1x Super Therm polymerase buffer (20 mM Tris-HCl, pH 8, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol), and 1 unit of Super Therm DNA polymerase enzyme (SR Products, UK). The PCR reactions were performed in a thermal cycler (Appligene) programmed for initial denaturation in 30 s at 94°C followed by 35 cycles of 25 s at 94°C, 45 s at 35°C, and 1 min at 72°C. Amplification was terminated by a final extension step of 5 min at 72°C. Amplification products were electrophoresed in 1.5% agarose gel, viewed on a UV transilluminator after staining with ethidium bromide (2 µg/ml), and photographed. Lambda DNA-EcoR I/Hind III double digest was used as a molecular size marker and run parallel to the amplified products.

Data analysis. RAPD bands were scored using a data matrix for the presence (1) or absence (0) of markers for the 19 primers that produced polymorphic bands in both broodstocks. Sizes in base pairs (bp) were inferred by comparison with lambda DNA-EcoR I/Hind III. The RAPD patterns of individuals were compared within and between broodstocks. A RAPD data matrix was used to determine the genetic similarity between individuals in terms of average band sharing frequencies (Nei and Li, 1979). The genetic similarity index (band sharing) was calculated as: $S_{xy} = 2(N_{xy})/(N_x + N_y)$, where N_{xy} is the number of bands present in both individuals, N_x is the number of bands in individual x, and N_y is the number of bands in individual y.

The Student's *t* test (Steel and Torrie, 1980) was performed to compare average genetic similarity between Seferihisar and Aliaga broodstocks.

Genetic distance was calculated as described by Lynch (1991), $D_{ij} = -\ln S_{ij}/(\sqrt{S_i} \times S_j)$, where D_{ij} is the approximate genetic distance between broodstocks i and j, S_{ij} is the average genetic similarity index between broodstocks i and j, S_i is the average similarity index in broodstock i, and S_j is the average similarity index in broodstock j. A dendrogram was constructed using Ward's method of hierarchical cluster analysis of JMP (SAS, 1996)

Results

Of the 40 primers, 25 revealed a pattern with scorable amplified primers in the Seferihisar broodstock and 28 revealed such a pattern in the Aliaga broodstock. Nineteen revealed patterns with scorable amplified primers in the both broodstocks (Table 1). Primers OPA-7, OPA-9, OPA-10, OPA-11, OPA-16, OPA-18, OPB-1, OPB-2, OPB-5, OPB-8, OPB-11, OPB-12, OPB-17, and OPB-18 exhibited the highest quality band patterns.

Genetic similarity averaged 0.466 within the Seferihisar broodstock (range 0.273-0.777; Table 2) and 0.617 within the Aliaga broodstock (range 0.420-0.770; Table 3), a significant difference of $p \leq 0.001$. The genetic similarity between Aliaga and Seferihisar broodstock averaged 0.420 (range 0.197-0.712; Table 4). The genetic distance between the two broodstocks was 0.245.

The dendrogram (Fig. 2) resulted in two main clusters: (a) nine fish from the Seferihisar stock (S1-3, S5-7, S9, S10, S15) and (b) all the Aliaga stock (A1-A15) plus six fish from the Seferihisar stock (S4, S8, S11-14).

Discussion

Seabream is one of the most important cultured fish species in the Mediterranean. Efforts to genetically improve its production traits (Knibb et al., 1997) may decrease genetic variation in cultured fish stocks as compared to wild stocks (Youngson et al., 2001). Palma et al. (2001), who studied the difference between cultivated and wild stocks, reported that rare alleles in the cultivated stocks disappeared as a result, in their opinion, of breeding techniques that rely on the same parental stock for several generations.

In our study, the average genetic similarity between the two stocks was 0.420, lower than the average within either stock. Bielawski and Puma (1997) used RAPD to detect genetic similarity within and between Atlantic coast striped bass (*Morone saxatilis*) and also found that the interpopulation average similarity (88.4%) was lower than the intrapopulation average similarity (93.8%). De Innocentiis et al. (2004) reported high polymorphism (7-38 alle-

Table 1. RAPD profiles of Seferihisar and Aliaga seabream broodstocks (19 primers).

Primer	*Sequence (5' – 3')*	*Seferihisar*		*Aliaga*	
		Bands (no.)	*Base pairs (range)*	*Bands (no.)*	*Base pairs (range)*
OPA-4	AATCGGGCTG	8	480-1433	7	512-1716
OPA-7	GAAACGGGTG	16	438-2283	12	438-2283
OPA-9	GGGTAACGCC	9	510-1270	8	510-1270
OPA-10	GTGATCGCAG	3	462-594	9	462-1032
OPA-11	CAATCGCCGT	12	461-1567	10	491-1567
OPA-13	CAGCACCCAC	3	590-804	5	590-1318
OPA-14	TCTGTGCTGG	5	555-944	4	587-944
OPA-18	AGGTGACCGT	11	497-1076	7	497-978
OPA-19	CAAACGTCGG	4	612-1345	12	506-1451
OPA-20	GTTGCGATCC	13	482-1592	13	482-1592
OPB-1	GTTTCGCTCC	15	508-1936	15	465-1936
OPB-5	TGCGCCCTTC	11	619-1599	16	459-1599
OPB-6	TGCTCTGCCC	9	494-922	10	494-942
OPB-7	GGTGACGCAG	11	483-1362	11	483-1362
OPB-8	GTCCACACGG	10	468-1254	9	468-1254
OPB-11	GTAGACCCGT	15	523-2520	15	523-2520
OPB-12	CCTTGACGCA	10	453-1029	13	453-1811
OPB-17	AGGGAACGAG	12	456-1640	11	533-1640
OPB-18	CCACAGCAGT	8	583-1310	7	583-1310
Total	-	185	-	194	-

les/locus) for four microsatellite loci among gilthead seabream populations from the Atlantic Ocean, Mediterranean Sea, and Adriatic Sea, concluding that the Mediterranean population differed from the Atlantic and Adriatic populations.

Genetic diversity within the Seferihisar broodstock was greater than within the Aliaga broodstock. Seferihisar broodstock originated from two areas, which may explain the greater variation in Seferihisar broods.

The dendrogram shows no strong genetic differentiation between the two stocks as some of the Seferihisar specimens fell into the same cluster as the Aliaga broodstock. This suggests some genetic exchange between the Aegean Sea and eastern Mediterranean wild seabream populations.

The level of genetic diversity is an important consideration in establishing a broodstock for selective breeding because it provides an indication of the scope for selective progress (Silverstein et al., 2001). In this respect, because of its relatively greater genetic diversity, the Seferihisar broodstock could serve as material for selective breeding.

In conclusion, RAPD effectively determined the genetic diversity within and between two gilthead seabream broodstocks in Turkey. In this study, we worked on limited strains. Future studies should examine wild as well as cultivated strains.

Table 2. Genetic similarity within Seferihisar broodstock.

	S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*	*S12*	*S13*	*S14*
S2	0.411													
S3	0.391	0.541												
S4	0.352	0.524	0.638											
S5	0.327	0.342	0.457	0.423										
S6	0.396	0.273	0.324	0.389	0.374									
S7	0.387	0.516	0.486	0.450	0.364	0.401								
S8	0.460	0.525	0.564	0.780	0.375	0.351	0.532							
S9	0.494	0.327	0.398	0.383	0.371	0.403	0.490	0.482						
S10	0.398	0.367	0.515	0.376	0.337	0.287	0.460	0.460	0.518					
S11	0.387	0.464	0.451	0.590	0.325	0.265	0.521	0.659	0.455	0.380				
S12	0.460	0.515	0.584	0.777	0.384	0.378	0.499	0.764	0.450	0.385	0.667			
S13	0.451	0.483	0.541	0.674	0.387	0.412	0.477	0.689	0.398	0.442	0.562	0.760		
S14	0.401	0.427	0.641	0.720	0.381	0.357	0.428	0.718	0.390	0.482	0.563	0.750	0.672	
S15	0.404	0.396	0.590	0.421	0.356	0.246	0.390	0.470	0.381	0.523	0.392	0.447	0.523	0.499

Table 3. Genetic similarity within Aliaga broodstock.

	A1	*A2*	*A3*	*A4*	*A5*	*A6*	*A7*	*A8*	*A9*	*A10*	*A11*	*A12*	*A13*	*A14*
A2	0.588													
A3	0.536	0.631												
A4	0.595	0.634	0.655											
A5	0.546	0.561	0.604	0.627										
A6	0.497	0.552	0.694	0.642	0.636									
A7	0.553	0.594	0.577	0.670	0.628	0.680								
A8	0.587	0.665	0.701	0.660	0.711	0.728	0.670							
A9	0.516	0.485	0.566	0.575	0.586	0.637	0.661	0.663						
A10	0.612	0.642	0.696	0.694	0.694	0.736	0.706	0.793	0.655					
A11	0.539	0.517	0.627	0.554	0.551	0.558	0.464	0.550	0.420	0.590				
A12	0.504	0.581	0.635	0.675	0.680	0.701	0.730	0.770	0.693	0.755	0.517			
A13	0.504	0.565	0.666	0.716	0.627	0.663	0.656	0.674	0.622	0.737	0.555	0.737		
A14	0.507	0.550	0.644	0.640	0.583	0.625	0.612	0.676	0.560	0.735	0.515	0.711	0.751	
A15	0.430	0.519	0.592	0.569	0.515	0.606	0.589	0.627	0.585	0.636	0.548	0.658	0.601	0.610

Table 4. Genetic similarity between Seferihisar and Aliaga broodstocks.

	S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*	*S12*	*S13*	*S14*	*S15*
A1	0.287	0.318	0.416	0.454	0.257	0.324	0.380	0.471	0.326	0.332	0.363	0.466	0.442	0.436	0.345
A2	0.304	0.344	0.377	0.457	0.236	0.235	0.331	0.559	0.354	0.326	0.422	0.520	0.463	0.485	0.361
A3	0.362	0.369	0.406	0.524	0.317	0.251	0.343	0.527	0.407	0.293	0.443	0.592	0.472	0.493	0.368
A4	0.286	0.370	0.415	0.559	0.326	0.268	0.352	0.549	0.332	0.300	0.439	0.591	0.494	0.530	0.312
A5	0.367	0.409	0.466	0.582	0.318	0.299	0.443	0.538	0.414	0.304	0.476	0.634	0.495	0.526	0.299
A6	0.357	0.424	0.474	0.587	0.329	0.307	0.362	0.532	0.358	0.286	0.417	0.620	0.482	0.531	0.327
A7	0.299	0.376	0.436	0.556	0.310	0.269	0.351	0.544	0.262	0.274	0.387	0.576	0.509	0.487	0.278
A8	0.364	0.440	0.494	0.612	0.361	0.319	0.399	0.592	0.379	0.345	0.491	0.688	0.560	0.587	0.462
A9	0.354	0.451	0.529	0.561	0.349	0.290	0.416	0.528	0.329	0.310	0.440	0.616	0.529	0.496	0.375
A10	0.361	0.426	0.511	0.683	0.372	0.331	0.426	0.623	0.382	0.357	0.510	0.712	0.599	0.609	0.415
A11	0.327	0.283	0.351	0.408	0.228	0.197	0.295	0.437	0.325	0.254	0.329	0.443	0.393	0.414	0.293
A12	0.380	0.437	0.491	0.644	0.386	0.346	0.375	0.626	0.377	0.334	0.519	0.686	0.631	0.590	0.361
A13	0.303	0.407	0.507	0.600	0.364	0.226	0.341	0.579	0.356	0.340	0.491	0.646	0.530	0.605	0.363
A14	0.266	0.399	0.449	0.634	0.305	0.252	0.327	0.591	0.317	0.317	0.450	0.627	0.559	0.567	0.340
A15	0.302	0.358	0.418	0.539	0.308	0.226	0.333	0.487	0.297	0.259	0.429	0.541	0.513	0.509	0.333

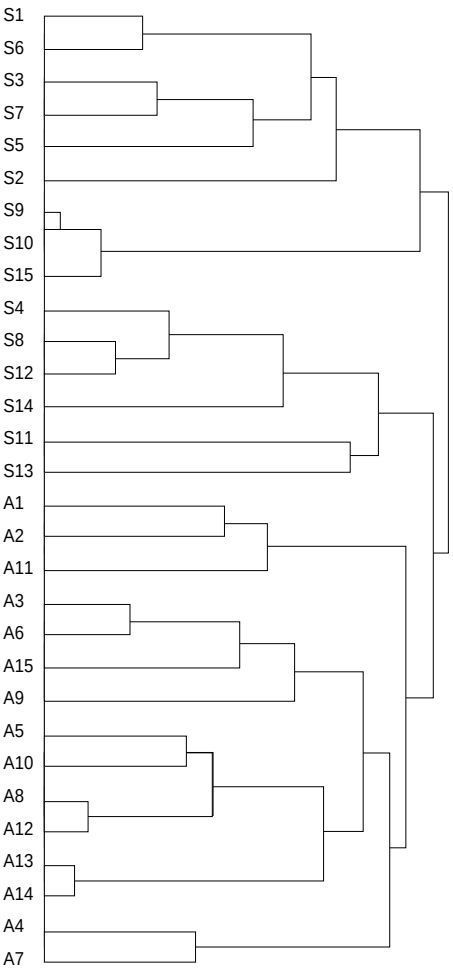

Fig. 2. Genetic relationship between Seferihisar and Aliaga broodstocks.

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