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PROOF RESEARCH PAPER

Evaluation of Genetic Diversity and Population Genetic Parameters of Farmed Turbot Species (*Scophtalmus maximus*) from France, Turkey, and Korea

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Abstract

Turbot (*Scophthalmus maximus*, Turbot) is a European flounder species, characterized by the absence of scales on the epidermis and the presence of small protuberances. Its capacity to be cultured effectively in fish farms at high densities makes it appealing in the aquaculture industry specifically in the UK, France and Spain. In recent years, development of a new strain by managing genetic resources is being intensively studied in Korea. It is a necessity in aquaculture to check the genetic diversity of a certain species in order to propagate better performing generations of its species. Microsatellite refers to 2-4 nucleotide repeats throughout genome with highly diverse repeat numbers, allowing of which to be used as biological marker for population identification and evaluation of genetic diversity. Population genetic analysis was carried out through the basis of 36 alleles which are different among the groups, presented by eleven markers that could be used in differentiating the genetic platforms of farmed turbot populations from Korea, France, and Turkey. The results obtained can be used in developing markers that are beneficial in identifying superior traits which in turn can be helpful in securing genetic diversity. In conclusion, this study identified genetic differences between farmed turbots in France and Turkey against the S. Korea. Based on the results, it can be implied that the genetic pool is capable of being expanded, and thereby maintenance of genetic diversity and improvement of excellent traits would be possible.

Keywords: Microsatellite marker, turbot, Genotyping, Genetic diversity, UPGMA.

Introduction

Turbot (*Scophthalmus maximus*, Turbot) is a laterally flattened fish (Bauchot, 1987; Larrazabal, 1992; Muss and Nielsen, 1999), that mainly thrives on gravel and sand covered bottom of shallow waters as deep as 20 ~ 70 m. In early 1970s, this species was adopted as an aquaculture candidate in Britain and France for its high economic value, high density culture tolerance and rapid growth. In 1970-72, French researchers successfully obtained eggs and fry from captive brood stock. Spain initially imported large quantities of eggs from France, but now they become the major producer. Turbot was first introduced to South Korea as fry from Scotland in February 1988, and now its production is about 300 tones, which allow South Korea to export turbot to countries in North America.

In order to continuously produce superior seeds of turbot, a lot of studies are being carried out on this species in China, Europe, especially Britain, France and Turkey. In general, cultured fish might have a small gene pool, because they are produced from a limited number of brood fish compared to natural populations. Offspring of generations that were artificially cultivated may produce seedlings that have reduced genetic diversity due to inbreeding and deliberate selection, this could lead to inbreeding depression. This can lead to genetic diseases that cause many problems in farming business of the species. Understanding the level of genetic diversity in turbot is therefore an important part of developing future aquaculture operations which requires continuous monitoring to preserve genetic resources (Hamrick & Godt 1996).

Analysis using microsatellite markers is widely used as a means of monitoring genetic diversity. Microsatellite refers to 2-4 nucleotide repeats found throughout the genome. It has a high degree of individual diversity and co-dominance, and is used for evaluation of genetic diversity and phylogenetic analysis in aquatic organisms. (Norris, Bradley & Cunningham 2000; Li, Park, Endo & Kijima 2004; Barroso, Hilsdorf, Moreira, Cabello & Traub-Cseko 2005). Microsatellite markers for turbot (*Scophthalmus maximus*) have been intensively developed and applied for studies of genetic diversity of the species. (Pardo *et al*, 2007; Gu, Zhao, Liu, Lu,

& Sun, 2009).

In this paper, we used microsatellite markers to compare the genetic diversity and divergence of the cultured French, South Korean and Turkish turbot stocks. The data obtained from this study provided the opportunity to evaluate current genetic status of the domesticated turbot stocks and it certainly will help to future studies for genetic improvement of its production efficiency.

Material and Methods

Turbot Samples and DNA Extraction

The samples used for this study were from aquaculture farms, but the Turkish samples were from the national research institute where they were collected from the Black Sea and domesticated. Therefore, the Turkish samples seemed, to some extent, from natural populations. The groups within a locality (ei., Korea 1-3) indicates the time differences for the collection. The DNA extracted from the tail fins of 1134 (Korea), 1014 (France), and 36 (Turkey) turbots was extracted with E.Z.N.A.® Mollusk DNA kit (Omega). The ethanol-immobilized samples were cut into the appropriate size, and the tissues were completely dissolved in Proteinase K (20 mg / ml) and lysis buffer at 60 ° C for more than 4 hours and then extracted according to the manufacturer's extraction method. After extracting, the presence of DNA was confirmed using 0.8% agarose gel. The integrity and quantity of DNA samples were determined by comparing their absorbance values at 260 and 280 nm, measured with a spectrophotometer (ND-1000) and the amount of DNA was mostly high concentration over 100 ng/㎕.

Microsatellite Analysis

Microsatellite markers were constructed using dinucleotide repeats at least 8 times and sufficient conserved sequences around the repetitive sites. A total of 11 loci were used for USC10, USC22, USC41, USC42, USC44, USC47, USC86, USC152, USC157, USC162 and USC272 (Table 1). PCR amplification reaction was performed using 10-100 ng of template DNA, 10X PCR Reaction Buffer, 10 mM dNTP, Taq DNA polymerase (5 U / μl), 11 multiplex PCR primers set was combined with a stepwise gene-amplification (touch-down PCR) method in which the annealing reaction temperature was set to 3 (56-58 ° C) so as to be universally usable in various devices (Table 2).

The amplified PCR products were diluted 30 times with TE buffer and diluted 10 times (1: 9) with GeneScan LIZ500 (Applied Biosystems, USA) size standard dissolved in Hi-Di Formamide (Applied Biosystems, USA). These samples were electrophoresed to be classified by size using an automatic sequencer. The standard allele ladder for each marker was prepared and scored using an analysis program such as GeneMapper version 4.0 (Applied Biosystems, USA), classified by size and type of markers, and collected and analyzed.

Table 1. Combine the Reagents

Component	Volume(㎕) for 1 Sample
10X PCR Reaction Buffer	1.5 ㎕
10mM dNTP mix	1.5 ㎕
Taq DNA polymerase(5U/㎕)	0.2 ㎕
Primer mix(11set)	8 ㎕
Template DNA(10~100ng/㎕)	Variable(1~3 ㎕)
Distilled water	Add up to 15 ㎕

Table 2. Conditions of Thermal-cycling

	Condition	
Initial Step	Pre-Denaturation	95□, 10min
1st Step	Melt	94□, 60sec
(5cycle)	Annealing	58□, 60sec
	Extend	72□, 60sec
2nd Step	Melt	94□, 60sec
(5cycle)	Annealing	57□, 60sec
	Extend	72□, 60sec
3rd Step	Melt	94□, 60sec
(25cycle)	Annealing	56□, 60sec
	Extend	72□, 60sec
1cycle	Final Extension	65□, 30min
	Final Step	8□ Hold

Statistical Analysis

Allele number per locus, allele frequencies, observed heterozygosities (Ho), expected heterozygosities (He), and PICs were used to analyze the genetic diversity of populations, and polymorphism information content were analyzed using the Cervus 2.0 program (Marshall *et al*., 1998). PIC indicates the polymorphic value of a marker within a population, using the number of detectable alleles and the distribution of their frequency in the population (Roychoudhury & Nei, 1988). In addition, the significance test for Hardy-Weinberg equilibrium (HWE) was performed using Population Genetic Analysis (Yeh, 1997).

Results and Discussion

Allele Frequency Analysis

Allele frequencies in 11microsatellite loci showed variations and also similarities among the three turbot populations (Figure 1). However, similar

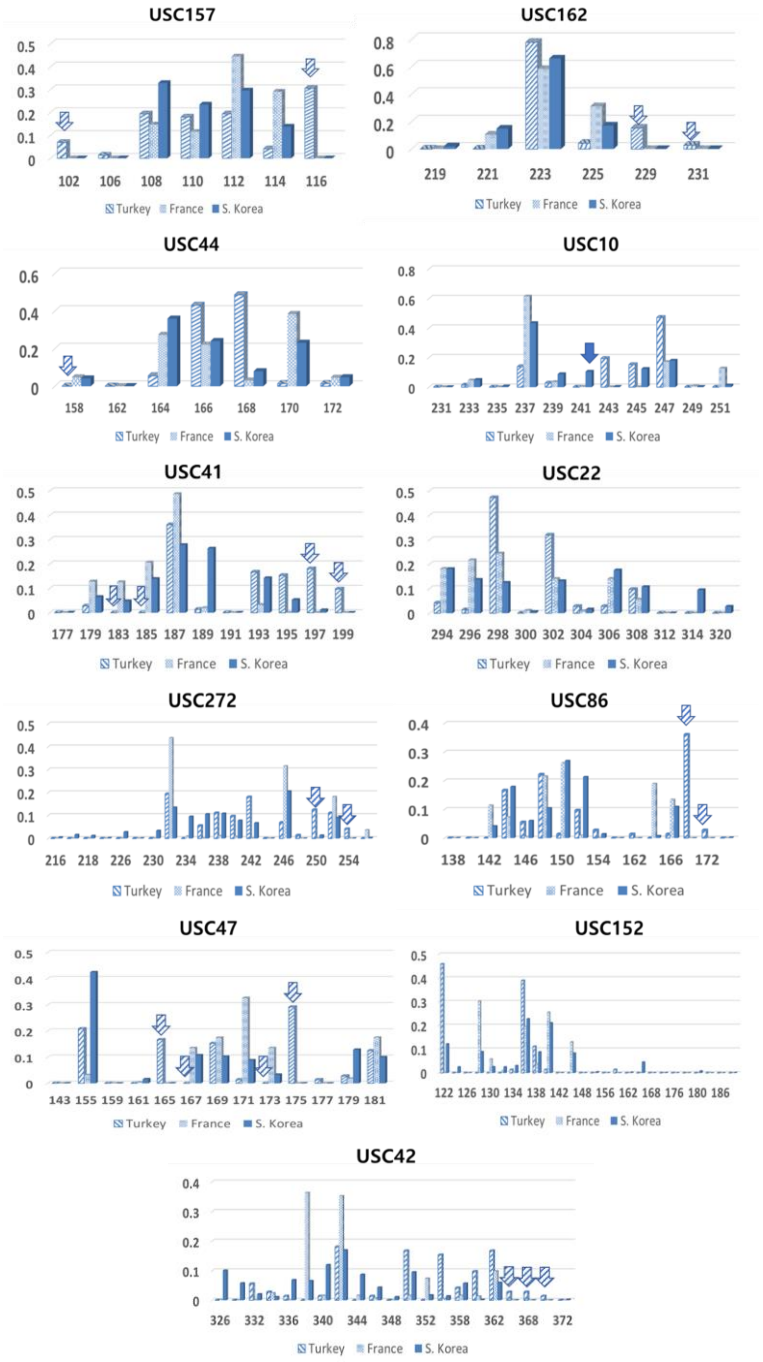

Figure 1. Allele frequency of 11 microsatellite markers among turbo groups. The arrows indicate alleles that are specific for Turkish turbot.

frequencies were also observed in some alleles in each representative turbot. Furthermore, alleles 116 and 170 from markers USC157 and USC 86 respectively showed high frequency in Turkish turbot, but they were low in French and S. Korea turbots. On the contrary, the frequency of alleles (USC44-158, USC10-241, USC41-183, and USC41-185) was very low in the Turkey turbot with 0.15 ~ 0.2. These results may imply that there has been a barrier for gene flow between groups of turbots from Turkey, France, and Korea. We presume, based on the results, that active genetic mutations or genetic drifts occur among the different groups and such genetic changes secure the diversity of their gene pools. Knowing that genetic recombination such as chromosomal changes actively occur in the system of the fish, it is essential to develop a way to secure the expression of exotic traits, to produce genetically superior groups and to prevent incapacitation of some traits through genetic exchanges.

Genetic Diversity Analysis between Group

Genetic diversity and population (French, Turkish and Korea) structure were analyzed through genotyping analysis using 11 developed microsatellite DNA markers. The PIC is used as a general indicator of each marker that indicates the genetic diversity of a particular population.

$$PIC_j = 1 - \sum_{i=1}^{n} P_i^2$$

j=the *j*th allele of the *i*th marker, n=the number of alleles at *j*th marker, p= allele frequency

FIS indicates the degree of intimacy of each individual, ranging from -1 to 1, with 0 being a stable state, indicating that the gene was fixed without reduction, while a value greater than 0 increased the risk of gene extermination, and a maximum of 1 means that the gene is completely *destroyed*. In other words, if there is a high degree of incorporation within a particular group, the group will rapidly become different from other groups making it genetically different. As a result, a marker with a low FIS value can show the genetic diversity of a group more accurately, this means that this specific marker has a high discrimination power.

The PIC values from each locus varies between populations (0.3-0.9) indicating the random loci were under indipendent selection pressure and seem ideal to use as a divergency index. There is no uniformed pattern of variation between populations (Table 3).

In this study, genetic diversity analysis results of the three groups of Turbot using microsatellite DNA markers (Turkey, Korea, France) are shown in Table 3. The number of alleles was 4 ~ 14 in Turkish, 4 ~ 24 in Korean, and 3 ~ 10 in French, turbot with average value of 7.6, 11.8 and 7.4, respectively, in which the Korean turbot showed the most number of alleles.

The heterozygosity ratio (Ho), which is the ratio of two alleles in a single individual, was lowest in Turkey, with an average of 0.730, 0.769 and 0.801 in Turkish, Korean and French turbots, respectively. The polymorphism index (PIC) was highest in Korea (0.766), and the FIS index, which is the index of the genetic status of the population, was very low (-0.128 ~ 0.026) in all groups. Although it is very likely that genetic characteristics will change drastically in a genetically stable state, the genetic diversity analysis and continuous analysis of additional samples are needed for more accurate results because the size of populations analyzed in the current Turkish population is small. Thus, further study might be needed.

Analysis of Genetic Distance and Relationship between Groups

Genetic linkage was determined by using the genetic analysis results of three domestic turbot groups, two groups imported from France, and three Turkish groups, and the genetic distance between groups using the formula of Nei's (1972). The estimated values for the genetic distance and similarity are shown in Table 4.

The highest genetic distance was observed between Korea and Turkey populations (averaged 1.138) and followed by between France and Turkey populations (averaged 0.987).

Based on Nei's genetic distance values, genetic distance between groups was calculated by UPGMA clustering method. As a result, Korean, French, and Turkish clusters were formed as follows. The French Turbot and the Korean Turbot are genetically close to each other, while the Turkey, France and Korea Turbot are genetically distant. Based on these results, it is expected that genetic diversity among groups will enhance the genetic diversity and excellence of traits.

Conclusion

Geographical distribution of organisms is one of the factors that suggest their genetic difference from other organisms even though they belong in the same species. In this study, the turbots in France, Turkey and S. Korea showed genetic diversity as was revealed from the genetic analysis using specific microsatellite markers.

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Table 3. Comparison of Genetic diversity among 3 groups of Turbot(Turkey, Korea, France)

Locus		Turkey	Korea	France	Locus		Turkey	Korea	France
USC10	k	6	4	4	USC41	k	7	10	9
	N	36	1134	1014		N	36	1133	1014
	HObs	0.667	0.734	0.927		HObs	0.778	0.773	0.842
	HExp	0.705	0.739	0.682		HExp	0.786	0.793	0.795
	PIC	0.655	0.690	0.627		PIC	0.745	0.771	0.767
	p	0.938	0.000	0.000		p	0.566	0.000	0.000
	Fis	0.056	0.008	-0.360		Fis	0.011	0.025	-0.059
	Ge. Di.	0.706	0.739	0.682		Ge. Di.	0.787	0.793	0.795
USC152	k	6	11	7	USC42	k	14	12	8
	N	36	1134	1014		N	36	1133	1014
	HObs	0.694	0.685	0.594		HObs	0.889	0.797	0.932
	HExp	0.635	0.727	0.575		HExp	0.883	0.852	0.813
	PIC	0.553	0.696	0.537		PIC	0.858	0.834	0.786
	p	0.414	0.000	0.000		p	0.012	0.000	0.000
	Fis	-0.096	0.058	-0.033		Fis	-0.006	0.064	-0.146
	Ge. Di.	0.634	0.727	0.574		Ge. Di.	0.883	0.852	0.813
USC157	k	7	4	3	USC44	k	5	11	8
	N	36	1134	1014		N	36	1131	1014
	HObs	0.861	0.541	0.679		HObs	0.611	0.848	0.884
	HExp	0.803	0.516	0.552		HExp	0.583	0.864	0.818
	PIC	0.761	0.472	0.476		PIC	0.484	0.848	0.792
	p	0.890	0.013	0.000		p	0.142	0.000	0.000
	Fis	-0.074	-0.049	-0.230		Fis	-0.049	0.018	-0.081
	Ge. Di.	0.802	0.516	0.552		Ge. Di.	0.583	0.864	0.818
USC162	k	4	9	6	USC47	k	8	19	7
	N	36	1134	1014		N	36	1132	1013
	HObs	0.389	0.789	0.645		HObs	0.889	0.921	0.751
	HExp	0.374	0.787	0.687		HExp	0.815	0.886	0.673
	PIC	0.338	0.757	0.647		PIC	0.776	0.876	0.614
	p	0.972	0.000	0.000		p	0.921	0.000	0.000
	Fis	-0.039	-0.003	0.061		Fis	-0.092	-0.040	-0.116
	Ge. Di.	0.374	0.787	0.687		Ge. Di.	0.814	0.886	0.673
USC22	k	7	19	13	USC86	k	10	24	10
	N	36	1130	1014		N	36	1082	1003
	HObs	0.583	0.910	0.959		HObs	0.778	0.669	0.766
	HExp	0.671	0.909	0.728		HExp	0.789	0.861	0.782
	PIC	0.608	0.901	0.686		PIC	0.749	0.846	0.748
	p	0.855	0.000	0.000		p	0.008	0.000	0.000
	Fis	0.133	-0.001	-0.317		Fis	0.014	0.223	0.020
	Ge. Di.	0.673	0.909	0.728		Ge. Di.	0.789	0.861	0.782
USC272	k	10	7	6	Average	k	7.6	11.8	7.4
	N	36	1134	1014		N	36.0	1128.3	1012.9
	HObs	0.889	0.787	0.831		HObs	0.730	0.769	0.801
	HExp	0.882	0.772	0.724		HExp	0.721	0.791	0.712
	PIC	0.856	0.734	0.677		PIC	0.671	0.766	0.669
	p	0.329	0.000	0.000		p	0.550	0.001	0.000
	Fis	-0.008	-0.020	-0.148		Fis	-0.014	0.026	-0.128
	Ge. Di.	0.882	0.772	0.724		Ge. Di.	0.721	0.791	0.712

k; number of alleles, N; number of samples, H(O); Observed heterozygosity, H(E); Expected heterozygosity, PIC; Polymorphic information content, p; probability of Hardy-Weinberg equilibrium, F_{IS}; inbreeding coefficient, Ge. Di.; Gene diversity

Table 4. Nei's (1972) genetic distance between each pair of turbot group

pop	France 1	France 2	Korea 1	Korea 2	Korea 3	Turkey 1	Turkey 2	Turkey 3
France 1	****	0.7526	0.4038	0.4297	0.5192	0.2148	0.4337	0.4615
France 2	0.2842	****	0.6899	0.4087	0.6272	0.2919	0.3772	0.5645
Korea 1	0.9067	0.3712	****	0.5371	0.75	0.2685	0.3036	0.5192
Korea 2	0.8447	0.8947	0.6216	****	0.5371	0.25	0.1615	0.4297
Korea 3	0.6554	0.4665	0.2877	0.6216	****	0.2685	0.347	0.5192
Turkey 1	1.5379	1.2312	1.3147	1.3863	1.3147	****	0.5249	0.4834
Turkey 2	0.8353	0.9749	1.192	1.8232	1.0585	0.6445	****	0.5639
Turkey 3	0.7732	0.5719	0.6554	0.8447	0.6554	0.727	0.573	****

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