Journal of Applied Biological Sciences 3 (1): 103-107, 2009
ISSN: 1307-1130, www.nobel.gen.tr

Population Genetic Structure of cobia, *Rachycentron canadum* Revealed by Microsatellite Markers

Mohammad Ali Salari - Aliabadi[1]* Sohrab Rezvani Gilkolaei[2] Ahmad Savari[1] Hossein Zolgharnein[1] Sayed Mohammad Bagher Nabavi[1]

[1] Department of Marine Biology, Khorramshahr University of Marine Science and Technology, P.O. Box: 669, Khouzestan, Iran

[2] Iranian Fisheries Research Institute, P.O. Box: 14155-6116, Tehran, Iran

*Corresponding Author
E-mail: salari1346@yahoo.com

Received: October 17, 2008
Accepted: January 03, 2009

Abstract

Information on the genetic structure of fish species is essential for optimizing fisheries management and stock improvement programs. Ten microsatellite loci were analyzed to study the genetic variation in six populations of the cobia (*Rachycentron canadum*). Seven of the ten loci analyzed were polymorphic in all the populations. Locus Rca 1B-H09 had the highest numbers of alleles (18), while the locus Rca 1B-A10 and Rca 1B-E08A had the lowest (14). All the studied populations deviated from Hardy–Weinberg equilibrium proportions at a number of loci, mostly due to the deficiency of heterozygosities. A moderate level of population differentiation (F_{ST}) was observed among populations; however, highest significant differentiation was between the Dayer and Pozm populations. The genetic distance computed by Nei between the Dayer and Beris populations was higher than the genetic distances between all other population pairs. The study revealed a relatively moderate level of genetic variation at microsatellite loci within and between cobia populations.

Keywords: PCR**,** Genetic variation, Polymorphism, Persian Gulf, Oman Sea

INTRODUCTION

Cobia, *Rachycentron canadum*, is an economically important, pelagic fish distributed in tropical warm waters worldwide [1]. It is a highly prized food and recreational trophy fish, and is considered a prime candidate for aquaculture [2]. Because of the popularity of cobia as a 'game' fish, methods to identify or distinguish products harvested in cobia aquaculture from 'wild' stocks will be needed in order to ensure legal sale and alleviate potential conflicts. Nuclear-encoded microsatellites are especially well suited for this purpose because of their codominant, Mendelian inheritance and their high levels of polymorphism [3]. Microsatellites have many applications in breeding programs and for assessing population structure of 'wild' populations as a means to improve assessment and allocation of resources [4]. The low variability at isozyme loci in the marine species, involving primarily diallelic polymorphism, reduces their sensitivity. In contrast, microsatellite DNA markers (one-to-eight-nucleotide tandem repeats, randomly distributed in the genome) have been found useful for detecting high levels of polymorphism and rare alleles. These markers are now widely used for the determination of genetic variation in wild and cultured fish populations [5-8].

Pruett [9] developed twenty nuclear-encoded microsatellites from a genomic DNA library of cobia and screened among a sample of 24 fish from the Gulf of Mexico. Renshaw [10] also developed 35 pairs of microsatellite markers for *R. canadum* and reported the numbers of alleles and heterozygosity observed in a single sample comprising 32 fish from the Gulf of Mexico. The aim of the present study was to assess the intra- and inter-population genetic variation in six populations of cobia in the northern coasts of Persian Gulf and Oman Sea using the microsatellite DNA markers developed by Pruett [9] and Renshaw [10].

MATERIALS AND METHODS

Experimental fish

184 specimens of *R. canadum* were collected from six zones (Booshehr, Dayer, Lengeh, Bandarabass, Pozm and Beris of Chabahar) between June and April of 2007 (Fig. 1). The zones are located within 89-451 km of each other in the northern coasts of Persian Gulf and Oman Sea. A small sample of the pectoral fin in the individuals were collected from 184 fish and preserved in 95% ethanol at 4°C. The sampling sites are shown in Fig. 1.

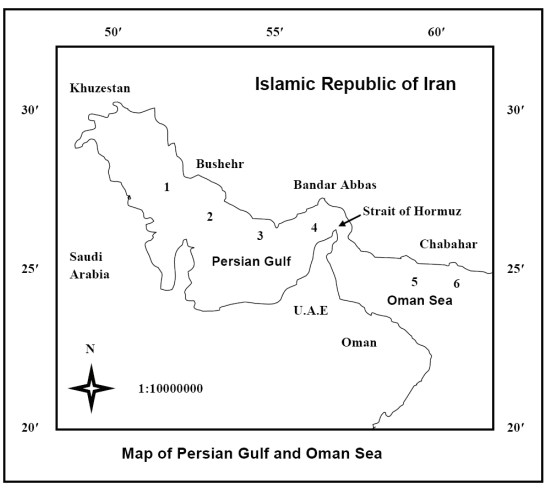


Figure 1. Map of localities sampled for cobia (R. canadum) in the northern coasts of Persian Gulf and Oman Sea, Iran. populations 1– 6 (1: Booshehr; 2; Dayer; 3: Lengeh; 4: Bandarabass; 5: Pozm; 6: Beris).

1-A04 developed by Pruett [9], were used in this study. The PCR conditions, especially the annealing temperatures, were optimized for the ten microsatellite loci as necessary to produce scoreable amplification products. The annealing temperatures varied from 55°C for Rca 1B-F07 and Rca 1B-E08B, 58°C for Rca 1B-F06, 60°C for Rca 1B-D09, Rca 1B-H09 and Rca 1-A04, 61°C for Rca 1B-A10 and 62.8 °C for Rca 1B-G10 to 63°C for Rca 1B-E02 and Rca 1B-E08A. PCR was performed in a 25 µl reaction volume containing 100 ng of template DNA, 2 µM of each primer, 0.4 mM each of the dNTPs, 1 unit of Taq DNA polymerase, 2 mM MgCl2 and 2.5 µl 10X reaction buffer. The temperature profile consisted of 3 min initial denaturation at 94 °C followed by 30 cycles of: 30 s at 94 °C, 45 s at the respective annealing temperature, and 1 min at 72 °C, ending with 10 min at 72 °C.

Electrophoretic separation of the amplified products and visualization

The PCR products were separated on a 8% denaturing polyacrylamide gel containing 19:1 acrylamide: bis-acrylamide and 5 M urea. Electrophoresis was conducted using a SequiGen sequencing gel electrophoresis system (BIO-RAD Laboratories, Hercules, CA). A pre-run of 30 min at 120 W was followed by a final run at 60 W until the loading buffer reached the bottom of the plate. DNA fragments were visualized by silver staining [12].

Scoring and statistical analyses

The size of each allele was estimated using the software DNAfrag, version 3.03 [13]. A genotypic data matrix was constructed for all loci. Fit of genotype data to Hardy–Weinberg proportions was estimated using the software POPGENE, (version 1.31) [14] with 1000 simulated samples. The GENALEX version 6 software package [15] was used for estimating allele frequencies and for applying the homogeneity test between populations. The dendrogram was constructed and drawn using MEGA version 4 [16].

RESULTS

Genetic variation

Of the ten microsatellite loci screened, seven were found to be polymorphic, while three locus, Rca 1B-D09, Rca 1B-E08B and Rca 1B-G10, were found to be monomorphic. Allelic variations at all loci in all populations are shown in Table 1. The locus Rca 1B-H09 had the highest number of alleles (18) (Table 1). The average number of alleles in the Bandarabass population was the highest observed (14.29), followed by that in the Lengeh population (13.43). The average number of alleles in the Beris population was the lowest observed (10.57). The observed heterozygosity in the Bandarabass population was higher than those of the other populations. The observed heterozygosities of all the populations at loci Rca 1B-A10, Rca 1B-E02, Rca 1B-E08A and Rca 1B-H09 were lower than the corresponding expected heterozygosities, while the observed heterozygosities at loci Rca 1B-F07 was higher than that expected (Table 1). The alleles Rca 1B-H09$_{218}$ and Rca 1B-H09$_{226}$ were found only in the Booshehr population, while alleles Rca 1B-F063$_{16}$ was found only in the Pozm population.

Table 1. Allelic variation at microsatellite loci in six populations of *R. canadum*. (N: Number of alleles per locus; N_e: Number effective alleles; H_o : observed heterozygosity; H_e : expected heterozygosity)

Population	Parameters	Locus						
		A10	E02	E08A	F06	F07	H09	A04
Booshehr	N	13	13	13	9	13	18	14
	N_e	8.3	11.1	7.9	6.1	8.3	11.6	8.7
	H_o	0.538	0.615	0.385	0.641	1.000	0.641	0.821
	H_e	0.880	0.910	0.873	0.837	0.879	0.914	0.886
Dayer	N	8	11	9	14	12	13	12
	N_e	4.7	7.4	5.9	8.6	8	8	6.9
	H_o	0.273	0.727	0.364	0.773	1.000	0.500	0.727
	H_e	0.788	0.865	0.830	0.883	0.875	0.875	0.854
Lengeh	N	14	12	13	8	16	16	15
	N_e	7.7	10.5	10.2	4.3	12.1	12.6	8.1
	H_o	0.625	0.625	0.313	0.781	1.000	0.750	0.813
	H_e	0.870	0.905	0.902	0.767	0.917	0.920	0.877
Bandarabas	N	14	12	13	15	16	15	15
	N_e	7.6	10.4	9	9	12.9	9.8	9.4
	H_o	0.659	0.634	0.366	0.659	1.000	0.732	0.854
	H_e	0.869	0.904	0.889	0.889	0.923	0.898	0.894
Pozm	N	11	13	11	8	11	13	12
	N_e	6.3	9.4	8.8	5.1	8.9	9.9	4.6
	H_o	0.533	0.700	0.267	0.600	1.000	0.767	0.733
	H_e	0.841	0.893	0.887	0.805	0.888	0.899	0.781
Beris	N	11	11	10	9	8	13	12
	N_e	7.4	8	7.3	7.1	6.6	8.4	6.5
	H_o	0.500	0.450	0.150	0.450	1.000	0.650	0.900
	H_e	0.865	0.875	0.864	0.860	0.848	0.881	0.845

Deviation from Hardy–Weinberg expectation

In 39 of 42 tests, significant deviations from the Hardy–Weinberg expectations were detected (Table 2). The test for fit to Hardy–Weinberg proportions revealed that the Booshehr, Dayer, Lengeh and the Bandarabass populations deviated at all the seven loci tested. The Pozm and the Beris populations deviated at 5 and 6 loci, respectively (Table 2). Except at loci Rca 1B-F07, in the majority of cases, the 1-Ho/He values were positive, meaning that all the populations were deficient in heterozygosities at a majority of the loci (Table 1). The deviations of the Rca 1B-A10 and Rca 1B-E08A loci from the Hardy–Weinberg expectation were larger in all the populations due to deficiency in heterozygosity in all the samples. However, it is problematic to assess the significance of departures from Hardy– Weinberg equilibrium when there are so many alleles at a locus, and results must be considered in this context.

Inter-population genetic structure

Population differentiation was modest, especially among populations from the same region systems. The population differentiation (F_{ST}) metric between the Dayer and the Pozm population was the highest (0.063) and significant among the population pair, while the F_{ST} metric between the Bandarabass and Lengeh population (0.000) was the lowest and not significant (Table 3). The estimated gene flow (N_m) value between the Bandarabass and the Lengeh population across all the studied loci was the highest, while the N_m value between the Dayer and the Pozm population was the lowest (Table 3).

Table 2. Deviation from Hardy–Weinberg genotype frequency expectations in six different populations of *R. canadum*. (χ^2 : Chi-square values; df: degrees of freedom)

Population	Parameters	Locus						
		A10	E02	E08A	F06	F07	H09	A04
Booshehr	χ^2	208	153	272	158	181	226	182
	Signif	***	***	***	***	***	***	***
	df	78	78	78	36	78	153	91
Dayer	χ^2	105	120	103	152	132	172	116
	Signif	***	***	***	***	***	***	***
	df	28	55	36	91	66	78	66
Lengeh	χ^2	210	120	240	99	184	157	256
	Signif	***	***	***	***	***	*	***
	df	91	66	78	28	120	120	105
Bandarabas	χ^2	275	154	287	248	242	136	349
	Signif	***	***	***	***	***	*	***
	df	91	66	78	105	120	105	105
Pozm	χ^2	100	109	168	36	102	82	91
	Signif	***	*	***	ns	***	ns	*
	df	55	78	55	28	55	78	66
Beris	χ^2	109	94	149	67	51	116	77
	Signif	***	***	***	**	**	**	ns
	df	55	55	45	36	28	78	66

* (P< 0.05, 5% significant to confidence level), ** (P<0.01, 1% significant to confidence level), *** (P<0.001, 0.1% significant to confidence level), ns (P>0.05, 5% insignificant to confidence level).

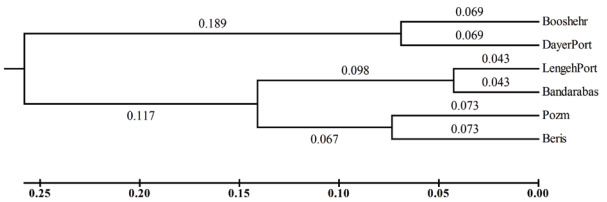
Table 3. Multilocus N_m (above diagonal) and F_{ST} values (below diagonal) between pairs of *R. canadum* populations across all loci. Genetic distances among the respective populations were

Populations	Booshehr	Dayer	Lengeh	Bandarabas	Pozm	Beris
Booshehr	****	108	14.2	11.9	5.5	5
Dayer	0.002	****	7.5	8.5	3.7	3.8
Lengeh	0.017*	0.032*	****	346	17	9.3
Bandarabas	0.021*	0.029*	0.000	****	16.1	16.4
Pozm	0.044*	0.063*	0.014*	0.015*	****	204
Beris	0.047*	0.062*	0.026*	0.015*	0.001	****

* (P<0.01, 1% significant to confidence level)

small. The genetic distances computed by Nei [17] between the Booshehr-Bandarabass and the Booshehr-Beris populations were the highest (0.258), while that of the Lengeh–Bandarabass populations was the lowest (0.043). The dendrogram based on genetic distance computed by Nei [17] (Fig. 2) showed two major clusters: the Booshehr and Dayer populations both were in one cluster, and the remaining four populations in the other cluster. The second cluster was further separated into two subclusters: the Lengeh and Bandarabass populations both were in one cluster and the Pozm and the Beris populations were in the other cluster (Fig. 2).

Figure 2. UPGMA dendrogram based on the genetic distance computed by Nei [17] between *R. canadum* populations, according to microsatellite DNA analysis.



DISCUSSION

Conservation of flanking regions is a general property of microsatellite loci and has been specifically reported in fishes [18]. Microsatellites have been isolated and characterized in a large number of fish species and have been used in a wide range of applications, as in evolutionary biology, population genetics and ecology [19].

Because the number of alleles observed in microsatellite loci is usually large and the frequency of each allele may be low, a large sample size is necessary for satisfying subsequent statistic analyses. The microsatellite loci used for cobia populations had enough genetic variation, with around 90 alleles across 7 polymorphic loci. Microsatellites allowed the identification of some unique alleles. In fact, as they were in low frequencies, it is probably that these unique alleles are rare alleles, once hyper-variable loci need a bigger sample to identify real unique alleles.

Frequencies of alleles in the Beris samples are comparable with those in the Booshehr population, except at one locus (Rca 1B-H09). It is likely that the Beris population had originated from the Booshehr and that it had lost some alleles during the course of fisheries and environmental management in the northern coasts of Persian Gulf and Oman Sea. The losses of alleles and heterozygosity may increase with bottlenecking and inbreeding through time in the natural stocks of cobia. Heterozygote deficiencies observed were different for different loci. Heterozygote deficiency can be interpreted as increase in homozygotes which might be a result of increased inbreeding. The significant heterozygote deficiency might reflect the fact that there is restricted gene flow between these populations. Although heterozygosities were low, there was enough variation present to examine any potential genetic differences among sample sites. Mean Heterozygosity per population was highly for each of these seven loci in the Iranian populations than in the northern Gulf of Mexico [9]. The mean Iranian population's value was 0.655 and the northern Gulf of Mexico value was 0.589.

Significant deviations from Hardy–Weinberg (HWE) expectations were observed in all six populations. Null alleles and homoplasy, frequently found in microsatellite loci, are likely causes for the H–W disequilibrium.

The partitioning of variability of populations seen after F-statistics comparisons with total types of markers shows that most of genetic variation is within populations. There was a high level of genetic differentiation among the six populations, with a highly significant overall F_{ST} value of 0.063 ($P < 0.01$). Based on Analysis of Molecular Variance (AMOVA) highest F_{ST} (0.063) was observed when comparing specimens from Dayer and Pozm zone (N_m =3.7). No spawning grounds of cobia was reported within the sampling zone except in the Dayer. Therefore, geological structures separate the Dayer from the other stocks and may limit the gene flow between the Dayer and any of the other five populations.

The genetic distances between different population pairs ranged from 0.043–0.258 which indicates that the genetic difference among the studied populations is pronounced. Nevertheless, higher genetic distances were observed between population pairs which are more geographically distantly located.

CONCLUSION

The present study showed that at least three different populations of *R. canadum* are found in the northern coasts of Persian Gulf and Oman Sea. These include the Booshehr region population, Bandarabass region population and the Chabahar region population. The present study also point out to the potentiality of the marker collections employed for a variety of purposes such as parentage testing, population monitoring and traceability in wild fish populations.

Acknowledgments

Funding for this project was provided by the Iranian Fisheries Research Organization (grant No. 2-019-200000-03-0000-86019), biotechnology library of the Caspian Sea ecological research institute and the Khorramshahr University of Marine Science and Technology. We also appreciate the comments of reviewers of this manuscript. The authors are grateful to Faramarz Laloi, Mohamad Javad Taghavi, Maryam Jaafarpoor, Fatemeh Khodabakhshi, Farzaneh Salari and Faezeh Salari.

REFERENCES

[1]Shaffer RV, Nakamura EL. 1989. Synopsis of biological data on the cobia *Rachycentron canadum* (Pisces: Rachycentridae). NOAA Technical Report NMFS. 82: 21-33.

[2]Benetti DD, Alarcon JF, Stevens OM, O'Hanlon B, Rivera JA, Banner-Stevens G. 2003. Advances in hatchery and grow out technology of marine finfish candidate species for offshore aquaculture in the Caribbean. Proceedings of the Gulf and Caribbean Fisheries Institute. 54: 473-487.

[3]Weber JL, May PE. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American journal of human genetics. 44 (3): 388-396.

[4]Garcia de Leon FJ, Canonne M, Quillet E, Bonhomme F, Chatain B. 1998. The application of microsatellite markers to breeding programmes in the sea bass, Dicentrarchus lab-

rax. Aquaculture. 159 (3-4): 303-316.

[5]Brooker AL, Cook D, Bentzen P, Wright JM, Doyle RW. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. Canadian journal of fisheries and aquatic sciences (Print). 51 (9): 1959-1966.

[6]DeWoody JA, Avise JC. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology. 56: 461-473.

[7]Norris AT, Bradley DG, Cunningham EP. 1999. Microsatellite genetic variation between and within farmed and wild Atlantic salmon (Salmo salar) populations. Aquaculture. 180 (3-4): 247-264.

[8]Was A, Wenne R. 2002. Genetic differentiation in hatchery and wild sea trout (*Salmo trutta*) in the Southern Baltic at microsatellite loci. Aquaculture. 204 (3-4): 493-506.

[9]Pruett CL, Saillant E, Renshaw MA, Patton JC, Rexroad CE, Gold JR. 2005. Microsatellite DNA markers for population genetic studies and parentage assignment in cobia, *Rachycentron canadum*. Molecular Ecology Notes. 5: 84–86.

[10]Renshaw MA, Pruett CL, Saillant E, Patton JC, Rexroad CE, Gold JR. 2005. Microsatellite Markers for Cobia, *Rachycentron canadum*. Gulf of Mexico Science. 23 (2): 248-254.

[11]Alam S, Popplewell A, Maclean N. 1996. Germ line transmission and expression of alacZ containing transgene in tilapia (Oreochromis niloticus). Transgenic Research. 5 (2): 87-95.

[12]Bassam BJ, Caetano-Anolles G, Gresshoff PM. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical biochemistry. 196 (1): 80-83.

[13] Nash, JHE. 1991. DNAfrag, Version 3.03. Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada.

[14]Yeh FC, Yang RC, Boyle T. 1999. POPGENE, Version 1.31: Microsoft Window-Based Free Ware for Population Genetic Analysis. http://www.ualberta.ca/~fyeh.

[15]Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288-295.

[16]Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Molecular Biology and Evolution. 24 (8): 1596-1599.

[17]Nei M. 1972. Genetic Distance between Populations. The American Naturalist. 106 (949): 283-292.

[18]Rico C, Rico I, Hewitt G. 1996. 470 million years of conservation of microsatellite loci among fish species. Proc. Biol. Sci., 263: 549-557.

[19]Dunham RA. 2004. Aquaculture and Fisheries Biotechnology: Genetic Approaches. 1st ed. CABI Pub, Wallingford, Oxfordshire, UK.