Int. J. Mar. Sci. Eng., 1(1), 69-73, *Autumn* 2011

Population genetic of *Eretmochelys imbricata* in two Islands in the northern part of the Persian Gulf using microsatellite markers

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Received 16 January 2011; revised 4 June 2011; accepted 17 August 2011

ABSTRACT: Nuclear markers such as microsatellites have allowed the identification of conservation and management populations of the Hawksbill turtles. In present study, eight microsatellite loci were studied. 60 samples of hawksbill turtles' flipper from Shidvar and Hormuz Islands have been surgically removed and preserved in 20% DMSO buffer. DNA was extracted using DNP KIT and amplified by PCR methods. The average number of alleles in Shidvar and Hormoz were 7 and 7.37 respectively with range of 7-13. The average expected and observed heterozygosity was 0.77 and 0.46 respectively. The linkage disequilibrium and deviations from Hardy-Weinberg equilibrium have been tested. The Fst values was 0.048, showing a significant difference between the two sites ($P<0.01$). The genetic distance between populations was found to be 0.27, which indicates that the genetic difference among the studied populations is pronounced. These results together with highly significant RST of genotypic differences between these pairs of samples support the existence of different genetic populations of Eretmochelys imbricata within the Iranian Islands of the Persian Gulf.

Keywords: *Hawksbill, microsatellite markers, Persian Gulf, Population genetic*

INTRODUCTION

The Hawksbill turtles, Eretmochelys imbricata, Linnaeus, 1766, belonging to the family Cheloniidae is widely distributed globally in subtropical and tropical waters including coastline of the Iranian Islands in the Persian Gulf. Total number of female turtles which are annually nesting in the Iranian Islands can be reached to 1000 (Rose and Barwani, 1982). The Hawksbills spend their first years of life in the pelagic habitats at the upper layer of the oceans. They nest on the insular and mainland sandy shores (Meylan and Donnelly, 1999). They are the only marine consumer whose diet predominantly comprises sponges (Castro and Huber, 2008). Genetic studies have advanced our understanding of marine turtle's biology. Since the advent of the Polymerase Chain Reaction (PCR) in the 1980s the use of microsatellites has become highly widespread in biological sciences. Microsatellites are clarified by the presence of a repetitive segment of DNA. These areas have high mutation rates that generate alleles of different lengths which can be useful as genetic markers for fine scales populations' resolution (Fitz Simmons et al., 1999). These molecular techniques provide new tools for peeking into the sex life of sea turtles (Dutton et al., 1991). As female turtles nest several times within a breeding season the study of genetic populations of them is complicated (Carr et al., 1978; Fitzsimmons et al., 1995; Mortimer and Bresson, 1994). The purpose of this study was to determine the genetic variation and the population structure of Hawksbill turtles in the Hormoz and Shidvar islands in the Persian Gulf.

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MATERIALS AND METHODS

Sampling and DNA Extraction: A total number of 60 samples of turtle's flipper were collected from E.imbricata specimens in the two different regions in northern part of the Persian Gulf including Hormoz and Shidvar Islands in the spring of 2009. Genomic DNA was extracted using DNP kit (Cinnagen). The quality and quantity of DNA were assessed by using 1% agarose gel electrophoresis.

PCR Amplification and Electrophoresis: Eight microsatellites loci were studied: Cc-2, Cc-13, Cc-28 (Monzon et al., 2008). And also HKB-17, HKB-26, HKB-29, HKB-30 and HKB-31 (Lin et al., 2008). The Polymerase Chain Reaction (PCR) was optimized for all microsatellites loci. Each PCR (total volume 25µl) was composed of 2.5µl 10x reaction buffer, 0.5µl dNTPs, 0.75µl MgCl2, 1µl of each primers, 1µl genomic DNA and 0.3µl of Taq DNA polymerase. PCR conditions were 95ωC for 1min followed by 30 cycles at 95ω C for 35s, at the respective annealing temperature for 35s, 72ω C for 35s and a final extension for 5 min at 72ω C. PCR products were size separated on 6% Polyacrylamide gels that were stained using silver nitrate. Electrophoresis pattern were analyzed using UVDoc software.

Microsatellite Analysis: Sizes of the PCR products were analyzed using GENALEX software (Peakall and Smouse, 2005). Each gel contained an allelic ladder (100bp) to assist in consistent scoring of alleles. Allele count and frequencies, Genetic diversity, expected and observed heterozygosity, Hardy-Weinberg equilibrium were computed. Genetic distance between two Populations was estimated from Nei standard formula (Nei, 1972, 1978). The genetic differentiation between two populations was evaluated by estimates of Fst value, using analysis molecular variance AMOVA (Analysis of Molecular Variance). In calculating P-values, 99 permutations were used. Specific markers were also identified for each of the populations.

Table 1. Variability of 8 microsatellite loci in two populations of Hormoz and Shidvar (NA: number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; loci in accordance with H-W unequilibrum (*P< 0.05 ; **P<0.01; *** P<0.001).

		HORMOZ	SHIDVAR
Cc-2	N.A	6	7
	HO	0.233***	0.923***
	HE	0.792	0.828
Cc-13	N.A	7	7
	HO	0.500***	0.385***
	HE	0.749	0.696
Cc-28	N.A	9	7
	HO	0.367***	0.077***
	HE	0.847	0.760
HKB-17	N.A	5	7
	HO	0.000***	0.154***
	HE	0.624	0.797
HKB-26	N.A	9	5
	HO	1.000***	1.000***
	HE	0.812	0.772
HKB-29	N.A	7	6
	HO	0.067***	0.192***
	HE	0.784	0.782
HKB-30	N.A	8	10
	HO	0.567***	0.846***
	HE	0.820	0.880
HKB-31	N.A	8	7
	HO	0.400***	0.692***
	HE	0.764	0.692
AVERAGE NO.OF ALLELES PER LOCUS		7.37	7
AVERAGE HO		0.39	0.53
AVERAGE HE		0.77	0.77

RESULTS AND DISCUSSION

The obtained results showed that all eight microsatellites were polymorphic (Table 1). The average allele number was 7 loci with the range of 5-10. The average number of alleles in Shidvar and Hormoz were 7 and 7.37 respectively and 6 specific alleles at a significant level ($P<0.05$) were also identified in Shidvar region. Within two populations, the average heterozygosity in Hormoz (0.39) was lower than the average heterozygosity in Shidvar (0.53). The deviations from Hardy-Weinberg equilibrium were also tested (Excoffier et al., 2005). Results have shown that all loci in two regions have deviations from Hardy-Weinberg equilibrium. The *FST*, as analyzed with *AMOVA*, showed a significant genetic differentiation among sites ($P < 0.01$). The population differentiation (F_{st}) value between Hormoz and Shidvar was 0.048 and the genetic distance within two populations was 0.27 which suggested that the populations diverged from each other.

The present research indicates that the Hawksbill turtles have declined during the last years in the northern part of thePersian Gulf as it was roughly estimated to number 1000 nesting females per years based on surveys conducted 27 years ago (Kinunen and Walczak, 1971).

High migratory behavior in marine turtles, also in Hawksbills, often consists of far trip entire ocean basins (Bolten et al., 1998). One of the biological characteristics of the Hawksbills adult females, that must be considered in the following discussion is that they return to the shores within the regions where they were born (natal homing) and therefore once a population is decreased it will not be reload by females from other rookeries within realistic time frame (Broderick et al., 1994; Broderick and Moritz, 1996; Bass et al., 1996; Bass, 1999; Bowen et al., 1996) and these must have cause demographic isolation between different populations and leading to the genetic differentiation in the nuclear and mitochondrial genomes (Schroth et al., 1999). In this study, the average number of alleles in Shidvar and Hormoz were 7 and 7.37 respectively which were lower than the average allele number, 11.63 of 40 samples of E.imbricata raised in Underwater World (Singapore) (Lin et al., 2008). The number of alleles or private alleles present in populations is useful for many conservation genetic applications. Perivate alleles which were identified showed that there is a native population in Shidvar which may have been caused for lack of human impact in the regions (Kalinowski, 2004). The low genetic distance and high gen flow was observed between Hormoz and Shidvar populations and therefore suggests that they originated from a same ancestor. Some factors such as sampling error, distance effects, behavioral effects (Shaw et al., 1999) migration and nesting ecology in sea turtles also in Hawksbills cause a pattern of gen flow and also create isolated populations in different geographic habitats. The estimated heterozygosity in two locations especially in Hormoz is lower than genetic variation for the hawksbills populations in other regions (Lin et al., 2008). The reduction of heterozygosity of this species in Iranian islands could be explained by many different factors as following: commercial exploitation which is the primary cause of the decline of Hawksbills, exploitation for eggs, loss of nesting and feeding habitat like destruction of coral reefs and sponges communities that Hawksbills rely on it as food resources (Lutcavege et al., 1997), mating and long life span that makes Hawksbills vulnerable in environment condition. Continue to the decline in quality and quantity of Hawksbills habitat will have reduced foraging opportunities of life and these may all to some extent design the genetic structure of populations (Donnelly and Townson, 2000; Garlach and Musolf, 2000; Palsson, 2000; Tiedemann et al., 2000). Loss of genetic variability recovers very slowly by some factors like mutations and migrations (Avise, 1994). The Hardy- Weiberg equilibrium showed that all loci in two regions have deviations from Hardy-Weinberg equilibrium which may have been caused by the presence of null alleles, high migratory and combination of populations, inbreeding, natural selection, population fragmentation, non-random mating and higher rates of mutation in microsatellite markers. In present study, Fst in all sampling sites was low but significant ($P\leq0.01$), suggesting at least two genetically differentiated populations and do not represent a single panmictic population.

CONCLUSION

As a conclusion, this study provides some information about the structure of genetic population and variability of Eretmochelys imbricata in Hormoz and Shidvar Islands in the Persian Gulf by microsatellite markers and showed that these markers have much ability for separating the populations of this species. These results may help the Department of Environment of Iran (DOE) to protect the genetic stock of these populations which are critically endangered.

ACKNOWLEDGMENTS

The authors would like to thank the Marine Research Bureau of Department of the Environment of Iran for financial support of this study.

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How to cite this article: (Harvard style)
Ghavam Mostafavi, P.; Shahnavaz, Sh.; Noroozi, M.; Fatemi, M. R.; Shahhosseiny, M. H.; Mahvari, A., (2011). Population genetic of Eretmochelys imbricata in two Islands in the northern part of the Persian Gulf using microsatellite markers. Int. J. Mar. Sci. Eng., 1(1), 69-73.