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Microsatellite Analysis of Genetic Diversity in Pekin (*Anas platyrhynchos*) and Muscovy (*Cairina moschata*) Duck Populations

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Abstract: In the present study the genetic structure of Pekin and Moscovy duck populations in north of Iran, Mazandaran province were analyzed using thirteen microsatellite markers. One hundred blood samples with equal number from each population were collected and DNA was extracted using modified salting out method. After Polymerase Chain Reaction (PCR), the PCR products were electrophoresed using 6% polyacrylamide gel. Four Out of 12 microsatellite markers were not amplified in any of both populations, three markers were monomorph and six markers generated polymorph bands. Some genetic parameters such as observed and effective allele number, mean of heterozygosity and genetic distance between two duck populations were estimated. The observed allele number in each locus was ranged from 1-4, effective allele number from 1-3.78, heterozygosity from 0-0.98 and the genetic distance between two populations was measured as 0.59 percentages. The low of genetic distance between two populations and the low level of mean heterozygosity index indicate that the genetic diversity is low in within and between populations. The low mean heterozygosity may be attributed to the low number of alleles present in the population, high level of inbreeding values because of small effective population size involved in studied duck breeding flocks. The analysis of Hardy-Weinberg equilibrium test showed that none of the microsattelite sites were at equilibrium. The obtained results at the present study indicated that characterization of genetic diversity by employing molecular tools is a prerequisite in developing strategies for conservation and utilization of duck genetic resources.

Key words: Microsatellite markers, genetic diversity, Pekin, Moscovy

Introduction

Recent advances in molecular technology have opened up completely new horizons in assessment of genetic variability at the DNA level. During the last two decades, different classes of molecular markers have become available for evaluation of genetic diversity within and between different livestock populations. These molecular markers that are revealing polymorphisms at the DNA level are key players in animal genetics and breeding programs. Microsatellites or Simple Sequence Repeats (SSR) are tandem repeated motifs of 1-6 bases that are found in all prokaryotic and eukaryotic genomes. Because of their high polymorphism, they have been extensively used in forensics, genetic mapping, population genetics, evolutionary studies (Sasazaki *et al*., 2004; Yoon *et al*., 2005). Microsatellite loci have gained widespread use due to their abundance in eukaryotic genomes, high polymorphism, codaminant nature, high reproducibility and relative ease of scoring (Vignal *et al*., 2002). It is not uncommon to find up to 10 alleles per locus and heterozygosity values of 60% in a relatively small number of samples (Goldstein and Polack, 1997). A large number of microsatellite markers have been mapped for various species, including humans, mice, cattle, sheep, pigs and poultry (Taylor *et al*., 1998; Groenen *et al*., 2000).

Fields and Scribner (1997) isolated and characterized novel waterfowl *(Somateria fischeri)* microsatellite loci where most of them were not highly polymorphic in Eider ducks *(Somateria mollissima)* population (Tiedermann *et al*., 1999). The first microsatellite markers developed have been reported by Maak *et al*. (2000) for Pekin and Moscovy duck. A total of 32 Pekin ducks comprising three strains and nine Moscovy ducks were investigated for polymorphisms. The average number of alleles was 2.9 and 3 in Pekin and Moscovy ducks, respectively. Williams *et al*. (2002) studied nuclear DNA-based markers for Mottled Ducks and determined levels of subdivision among populations in Florida. They screened 13 microsatellite primer pairs and identified six microsatellite loci that were variable in Mottled Ducks. These markers revealed a low level of genetic differentiation and a high level of genetic exchange among four Mottled duck sub populations within Florida. Their analysis of the Florida Mottled duck population indicated high levels of heterozygosity and no evidence of genetic subdivision among breeding units. Paulus and Tiederman (2003) have been developed and

Table 1: Primer sequences used for amplification of 12 microsatellite loci in Pekin and Muscovy duck populations in north of Iran

Locus	Sequences (5'-3')	Locus	Sequences (5'-3')
APHO1F	TACCTTGCTCTTCACTTTCTT	SMO6F	GGGGTGGGAAAGAAGCAGTTTAG
APHO1R	GTATGACAGCAGACACGGTAA	SMO6R	TCCTGGGACTTTGAAAGTGGCTC
APHO7F	ACATCTTTGGCATTTTGAA	SMO7F	TTTTCACCCAGTTCACTTCAG CC
APHO7R	CATCCACTAGAACACAGACAT T	SMO7R	GATTCAAATTTGCCGCAGGATTA
APHO9F	GGATGTTGCCCCACATATTT	SMO8F	TGCCTTATAGGATGTCACTCTTC
APHO9R	TTGCCTTGTTTATGAGCCATTA	SMO8R	AAAATACTATGCTCGTTTCAAAA
APH11F	GGACCTCAGGAAAATCAGTGTA	SMO11F	AAATCAACCAAAGAGGCATAG CC
APH11R	GCAGGCAGAGCAGGAAATA	SMO11R	GCAGTTGTTTTGGAGGACAGACA
APH14F	GAATAAAGTAACGGGCTTCTCT	SMO12F	CCTGGTGGGATAGGTTTAAAATG
APH14R	CTGCTTGGTTTTGGAAAGT	SMO12R	TGTTCATCAAAAGCAGAGAGGGG
SMO1F	CTTAAGGTATTGTGCTTTATA	SMO13F	ACCATCTTCCTTTCCTCCCAACC
SMO1R	TGGTCCAAAGGGTGTTCTGAGAA	SMO13R	GGGCTTGAGGCATACACTCCCTA

characterized polymorphic autosomal microsatellite loci for the Eider duck (*Somateria mollissima*) and their cross-species among waterfowl species *(Anatidae*). These microsatellite markers showed Mendelian inheritance and no linkage disequilibrium between any pair of loci. They have been observed between 2 and sixteen alleles per locus, an expected heterozygosity between 0.31 and 0.97 and an observed heterozygosity between 0.14 and 1.00 (Paulus and Tiederman, 2003). The genetic variation has been assessed among 225 Mottled and Mallards ducks using five microsatellite loci (Williams *et al*., 2004). In this study only 3.4% of mallards were inferred to have been hybrids, suggesting asymmetric hybridization. Populations from different geographic areas within Florida exhibited hybridization rates ranging from zero to 24%. A genetic linkage map for duck was developed within a cross between two extreme Peking duck lines by linkage analysis of 155 polymorphic microsatellite markers. A total of 115 microsatellite markers were placed into 19 linkage groups. The sex-averaged map spans 1353 cM, with an average interval distance of 15 cM. The male map covers 1415 cM, whereas the female map covers only 1387.6 cM (Huang *et al*., 2006). Up to now, no genetic data has been generated for duck populations in Iran. This is the first report on genetic diversity verifications based on tests with DNA markers in Pekin and Muscovy duck populations in Iran. Our specific objectives were to (1) assess genetic diversity within and among Pekin and Muscovy duck populations in north of Iran and (2) to determine the allelic patterns of microsatellite markers in the population studied.

Materials and Methods

Samples collection and DNA preparation: Venous blood samples were collected from 100 ducks of both populations into 3 ml tubes containing EDTA as anticoagulant agent. Genomic DNA was isolated from 1 ml blood aliquots by a standard procedure using proteinase K digestion, phenol chloroform extraction and ethanol precipitation method (Miller *et al*., 1988). The quantity and quality of the extracted DNA was determined by a spectrophotometric method based on absorbance at 260 and 280 nm respectively. The 12 microsatellite primers of specific sequence were tested for their potential use in the amplification of scorable bands (Table 1).

PCR conditions: Polymerase Chain Reactions (PCR) were performed in a final volume of 25 µl containing 15 ng of genomic DNA, 200 µM of each dNTP, 0.5 µM of each forward and reverse primers, 2.5 mM of $MgCl_2$, 1 unit of Taq DNA polymerase and 1X PCR reaction buffer. Negative control (lacking DNA) was set up for each reaction master mix to check for DNA contamination. The amplification was carried out in a thermocycler (Biometra), using the following conditions: an initial denaturation step at 95°C for 5 min followed by 35 cycles of 95°C for 1 min, annealing at 45-57°C for 45s, extension at 72°C for 1 min and a final extension of 5 min at 72°C. The PCR products were electrophoresed on 6% poly acryl amide gel and visualized after silver staining. The internal size standard was used for sizing of alleles.

Statistical analysis: To characterize locus and population genetic variation, the following models were used to calculate the observed (*Na*) and effective (*Ne*) allele numbers (Kimura and Crow, 1964).

$$Na = \frac{1}{x},\ Ne = \frac{1}{\sum xi^2}$$

The observed (H_O) and expected heterozygosity (H_E) was calculated using the following models (Hedrick, 1999). Test of genotype frequencies for deviation from Hardy-Weinberg Equilibrium (HWE) were carried out using the Markov chain method (Guo and Thompson, 1992).

$$H_O = \frac{\sum Nij}{N},\ H_E = 1 - \sum_{i=1}^{n} Pi^2$$

The Nei's standard genetic distance (*Ds*) was used to calculate the genetic dissimilarity between two populations (Nei, 1978).

Table 2: Allelic patterns of microsatellite loci in Pekin and Muscovy duck populations in north of Iran

	Pekin			Muscovy		
Name of locus	Total No. of alleles	Size of alleles	Frequency of alleles	Total No. of alleles	Size of alleles	Frequency of alleles
APHO1	2	-	-	3	192	0.032
		198	0.356		194	0.649
		200	0.644		202	0.319
APHO7	4	224	0.023	4	216	0.234
		232	0.227		236	0.298
		236	0.435		248	0.245
		282	0.326		260	0.223
APHO9	4	102	0.239	4	094	0.267
		110	0.348		108	0.289
		112	0.228		112	0.144
		126	0.185		130	0.300
APH11	2	193	0.50	4	191	0.092
		205	0.50		193	0.347
		-	-		205	0.429
		-	-		238	0.133
APH14	2	158	0.500	2	155	0.500
		178	0.500	175	0.500	
SMO1	-	-	-	-	-	-
SMO6	2	128	0.66	1	116	100
		140	0.34			
SMO7	1	188	100	1	188	100
SMO8	1	95	100	1	95	100
SMO11	2	182	0.50	-	-	-
		180	0.50			
SMO12	-	-	-	-	-	-
SMO13	2	195	0.59	2	195	0.54
		225	0.41	225	0.64	

$$Ds = -Ln(I), \; I = \frac{Jxy}{\sqrt{JxJy}}$$

Dendrogram was constructed using UPGMA method and POPGENE computer program was used to construct the tree from the distance matrix (Yeh *et al.*, 1999).

Results and Discussion

In the present study 10 out of 12 microsatellite markers were chosen for further analysis on the basis of diagnostic and scorable bands. Table 2 represents the observed allele numbers, size range and frequency in 10 microsatellite loci in Pekin and Muscovy duck populations. In total, 44 alleles were detected at 10 microsatellite loci typed in 100 individual ducks. The mean number of alleles per locus was 4. The markers SMO1 and SMO12 did not amplify in any of Pekin or Muscovy populations. In a similar study, involving Pekin, Muscovy, Shaldoc and Squter ducks (Paulus and Tiederman, 2003), SMO12 marker did not amplify in any of populations but SMO1 amplified only in Shaldoc and Squter. In our study we found tow specific bands (182 and 205bp) for SMO11 marker only in Pekin population, while Paulus and Tiederman (2003) found one band with the size of 282bp. In our study, the SMO7, SMO8 and SMO13 loci were monomorphic in both populations, while SMO6 showed monomorphic band only in Muscovy population. The microsattelite SMO11 marker has only amplified in Pekin population. At the present study we have found two specific bands (182 and 205 bp) for SMO11 marker in Pekin population. Paulus and Tiederman (2003) found one band with the size of 282 bp in this breed. Table 2 represents the statistics of allelic variation (total number of alleles, range of allele size and allele frequencies) in 10 microsatellite loci analyzed. The number of alleles identified at each locus for each population is considered to be a good indicator of genetic variability. Compared to previously published data in duck populations (Maak *et al.*, 2000; Williams *et al.*, 2002; Paulus and Tiederman, 2003), results of the present study revealed equal or much lower microsatellite allele variation in Pekin and Moscovy duck populations. Using Chi-square test for each locus at the present study did not show any deviation from HWE in both populations.

Table 3 summarized the results of analysis for genetic parameters for each locus across the two populations. Effective number of alleles ranged from 1 at SMO7 and SMO8 to 3.95 at APOH7 locus. The mean observed and effective allele numbers ranged from 2.22±0.1.03 to 2.029±0.82 in Pekin and 2.44±1.33 to 2.18±1.15 in Muscovy population, respectively (Table 3). Maak *et al.* (2000) reported an average allele numbers of 2.9 to 3 in

Table 3: Observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) in Pekin and Moscovy duck populations in north of Iran

	Pekin				Muscovy			
locus	Na	Ne	Ho	He	Na	Ne	Ho	He
APHO1	2	1.85	0	0.46	2	1.91	0.15	0.48
APHO7	4	2.91	0	0.66	4	3.95	0.53	0.75
APHO9	4	3.78	0.98	0.74	4	3.77	0.73	0.74
APH11	2	2	1	0.51	4	3.03	0.61	0.67
APH14	2	2	1	0.51	2	2	1	0.51
SMO1	-	-	-	-	-	-	-	-
SMO6	2	1.94	0.48	0.45	2	1.98	0	0
SMO7	1	1	0	0	1	1	0	0
SMO8	1	1	0	0	1	1	0	0
SMO11	2	2	1	0.50	-	-	0	0
SMO12	-	-	-	-	-	-	-	-
SMO13	2	1.94	0.82	0.49	2	1.98	0.92	0.50
Mean±SE	2.2±0.3	2.03±0.2	0.53±0.18	0.43±0.12	2.44±0.3	2.18±0.11	0.44±0.17	0.41±0.14

Table 4: Genetic distance between Pekin and Moscovy duck populations in north of Iran

Population	Pekin	Moscovy
Pekin	-	0.598
Moscovy	0.598	-

Pekin and Moscovy duck populations, respectively. In our study the total heterozygosity was moderate and ranged from 0.53 in Pekin to 0.44 in Moscovy duck population (Table 3). The maximum observed heterozygosity values were found for APHO9 loci in both populations. The low mean heterozygosity may be attributed to the low number of alleles present in the population, high level of inbreeding values because of small effective population size involved in breeding flocks. The other factors can also cause a lack of heterozygotes in a population (Jordana *et al*., 2000). First, the locus can be under selection, the genetic hitchhiking effect with some morphological or productive traits of selective interest. Second, null alleles (nonamplifying alleles) may be present which lead to a false observation of excess homozygotes. Third, the presence of population substructure may lead to Wahlund's effect. Table 4 shows the genetic distance between tow populations. The calculated genetic distance between these two populations was 0.598 and they are categorized in two separate groups. In conclusion, the characterization of genetic diversity by employing molecular tools is a prerequisite in developing strategies for conservation and utilization of duck genetic resources. We hope that this information will significantly contribute to the establishment of a sensible genetic preservation strategy for these populations.

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