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Original Contribution

STUDY OF GENETIC DIVERSITY OF DASHTIARI, KHAZAK AND ZABOL CHICKENS USING MICROSATELLITE MARKERS

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ABSTRACT

The genetic variability of three native chicken populations (Dashtiari, Khazak and Zabol) derived from Sistan and Baluchistan province in Iran was evaluated with 10 microsatellites markers (MCW5, MCW16, MCW18, MCW34, MCW39, ADL225, ADL262, ADL185, ADL136, ADL210). The mean heterozygosity was 0.77, 0.79 and 0.52 for Khazak, Dashtiari and Zabol chickens, respectively. The highest PIC was 0.81 in ADL225 and ADL136 at Dashtiari and Khazak chickens. The results of the heterozygosity were consistent with polymorphism information content (PIC). The genetic information in the current investigation will be used in conservation and improvement program of three native chickens.

Key words: Genetic diversity, Microsatellite marker, Dashtiari, Khazak, Zabol

INTRODUCTION

The determination of genetic variation based on DNA markers provides the best available objective information, and in the absence of other supporting data genetic distance analysis allows a ranking.

As a result of many years of domestication and breeding, a wide range of chicken breeds exist today. However, an increasing number of local chicken breeds are under threat of extinction, and valuable genotypes and traits are at risk of being lost (1).

In poultry different genetic marker systems have successfully been used for estimation of genetic characterization of chicken breeds and populations including: protein polymorphisms (2), blood protein polymorphisms (3), DNA fingerprinting (4), RAPD (random amplified polymorphic DNA, 5, 6), and microsatellites (7, 8, 9, 10, 11 and 12). Presently, the determination of heterozygosity and genetic distance based on microsatellite analyses is regarded as the most convenient tool and many microsatellite loci are available in chicken. Microsatellite markers are widely analyses, because they are easy to use and can provide an abundance of polymorphic information (13).

Poultry production is an important livestock sector in tropics contributing to a high proportion of human supply of animal protein through meat and eggs. It is especially favorable to the smallholder systems of developing countries of tropics due to low capital investment, high cost efficiency, flexible production systems and low production risk. Despite the importance of local chicken in human supply, information is scarce on their genetic make up with respect to performance, adaptability, resistance, genetic variability and genetic relationships.

The aim of our project was to evaluate the genetic variability of chicken populations sampled in Sistan and Baluchestan province of Iran and to measure all the genetic parameters the chicken populations. The results should be useful in order to provide the information for breeding programs.

MATERIALS AND METHODS

Samples

In total, DNA samples of 105 chickens were available for microsatellite genotyping. The blood samples were collected from 35 birds

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from each of the three native chickens of Sistan and Baluchestan province in Iran that was sampled from institute of special animal and in different villages' zones within this province. The populations represent well defined native breeds kept at the experimental station of special animal institute Zabol University.

DNA extraction

Venous blood was collected from ulnar vein of each individual, with EDTA as an anticoagulant.

DNA was isolated using phenol-chloroform method with modifications and 10 microsatellite primer pairs were selected for the present study **(Table 1)**.

Microsatellite Markers

Ten pair highly polymorphic microsatellite markers were chosen based on their genomic location **(Table 1)**.

PCR reactions

PCR amplifications were carried out in 25 μ_L reactions containing 50 ng of genomic DNA in a reaction mixture containing 1.5 mM $MgCl_2$, 200µM dNTPs, 0.50 µM of each primer and 1 U taq polymerase. The PCR amplification programme performed on Eppendorf Mastercycler Gradient consisted of an initial denaturation temperature of 95 C for 5 min, then 35 cycles at 94 °C for 45s, 50-64 °C for 1 min depending on the primer pair used and 72 °C for 1 min **(Table 1)**. Final extension was carried out at 72 °C for 15 min. PCR products were separated on 8% denaturing polyacrylamide gels.

Statistical analysis

Measurement of genetic variation within populations in order to estimate genetic variation within populations, heterozygosity and its total variation were calculated per strain per marker (14). Allele frequencies obtained from the microsatellite were used to calculate PIC values (polymorphic information content) in order to measure the degree of information obtained by microsatellite (15). Heterozygosity estimates based on genotyping results at all loci were checked for deviation from Hardy-Weinberg equilibrium with the software package GENEPOP V1.2 (16).

Table 1 *Characterization of 10 microsatellites*

Locus	Chromosome	Primer sequence 5'-3'
MCW0005	4	ACCTCCTGCTGGCAAATAAATTGC TCACTTTAGCTCCATCAGGATTCA
MCW0016	3	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG
MCW0018	1	GGAATTTGAACACCTGAGATTTCC CACTATGTTTATGGCAAACTCCTG
ADL0210	11	ACAGGAGGATAGTCACACAT GCCAAAAGATGAATGAGTAC
ADL0225	13	CCAAAAAGCTGTATCACCTT GCCTGTTGTAAACCACCTGA
MCW0034	2	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG
MCW0039	2	CATTGGACTGAGATGTCACTGCAG ACATTTGTCTAATGCTACTGTTAC
ADL0136	9	TGTCAAGCCCATCGTATCAC CCACCTCCTTCTCCTGTTCA
ADL0185	2	CATGGCAGCTGACTCCAGAT AGCGTTACCTGTTCGTTTGC
ADL0262	23	GTGCAGACACAGAGGGAAAG TCACATGCACACAGAGATGC

RESULTS

For all loci in three populations, significant deviation from Hardy-Weinberg equilibrium was found. Number of alleles, heterozygosity and effective number of alleles for the three native chickens are given in **table 2**. All the loci were polymorphic and the number of alleles varied between 2 (ADL262 in Khazak) and 10 (ADL225 in Dashtiari), with generally little difference between the native chickens. We found 52 alleles in Zabol, 50 in Khazak and 64 in

Dashtiari **(Table 2)**. The effective number of alleles ranged from 1.99 (ADL262) to 5.98(ADL225 and ADL136) in Khazak, from 2.77 (ADL262) to 7.43 (ADL225) in Dashtiari and from 3.71 (MCW39) to 6.22 (ADL136) in Zabol chicken. The mean effective number of alleles was highest in Dashtiari (5.09) and in Zabol and Khazak chicken was 4.69 and 4.50 respectively. Three populations showed heterozygosity and there was little difference in genetic variability of the populations. The mean heterozygosity was 0.77, 0.79 and 0.52 for Khazak, Dashtiari and Zabol chicken, respectively. The results show that heterozygosity is lower for the Zabol chickens. The polymorphism information content (PIC) ranged from 0.50 (MCW16 in Dashtiari) to 0.81 (ADL225 in Dashtiari and ADL136 in Zabol). PD content for three populations was high, ranged from 0.98 to 0.99. Also, the Shannon Index value was the lowest for ADL262 in Khazak (0.6897) and the highest for ADL225 in Dashtiari (2.0958) **(Table 3)**.

Table 2. *Genetic parameters measured in the Sistan and Baluchestan chicken native with 10 microsatellite loci*

locus	Observed features	Populations		
		Zabol	**Khazak**	**Dashtiari**
MCW5	**Allelic number(N_a)**	4	5	7
	H	0.522	0.8122	0.8213
	N_e	3.92	4.90	5.35
MCW16	**Allelic number(N_a)**	5	4	4
	H	0.667	0.7367	0.6919
	N_e	4.63	3.59	3.17
MCW18	**Allelic number(N_a)**	6	5	5
	H	0.520	0.7918	0.7918
	N_e	4.70	4.49	4.63
ADL210	**Allelic number(N_a)**	5	5	6
	H	0.304	0.8018	0.8001
	N_e	4.83	4.55	4.85
ADL225	**Allelic number(N_a)**	6	8	10
	H	0.385	0.8746	0.8881
	N_e	5.59	5.98	7.43
ADL185	**Allelic number(N_a)**	5	4	8
	H	0.607	0.8088	0.8481
	N_e	4.67	3.87	5.04
ADL262	**Allelic number**	4	2	5
	H	0.680	0.7320	0.7331
	N_e	3.94	1.99	2.77
ADL136	**Allelic number(N_a)**	7	7	8
	H	0.440	0.8711	0.8824
	N_e	6.22	5.98	7.33
MCW39	**Allelic number(N_a)**	4	6	6
	H	0.566	0.8442	0.8372
	N_e	3.71	5.83	5.87
MCW34	**Allelic number(N_a)**	6	4	5
	H	0.480	0.7519	0.7818
	N_e	4.68	3.82	4.35
Mean	**Allelic number(N_a)**	5.2	5	6.4
	H	0.517	0.7696	0.7936
	N_e	4.69	4.50	5.09

Table 3. *Measurement of PD, PIC and Shannon Index in three native chickens*

Locus		Zabol	Khazak	Dashtiari
MCW5	**PD**	0.992	0.9934	0.9947
	PIC	0.7	0.71	0.74
	Shannon Index	1.3761	1.5994	1.7705
MCW16	**PD**	0.992	0.9927	0.9929
	PIC	0.74	0.55	0.50
	Shannon Index	1.5671	1.3178	1.0324
MCW18	**PD**	0.993	0.9926	0.9883
	PIC	0.77	0.68	0.69
	Shannon Index	1.6386	1.5510	1.5765
ADL210	**PD**	0.993	0.9940	0.9881
	PIC	0.75	0.69	0.70
	Shannon Index	1.5922	1.5580	1.6595
ADL225	**PD**	0.996	0.9943	0.9920
	PIC	0.79	0.80	0.81
	Shannon Index	1.7537	1.9096	2.0958
ADL185	**PD**	0.993	0.9830	0.9926
	PIC	0.75	0.69	0.78
	Shannon Index	1.5705	1.3690	1.8738
ADL262	**PD**	0.993	0.9942	0.9930
	PIC	0.07	0.56	0.58
	Shannon Index	1.3788	0.6897	1.3657
ADL136	**PD**	0.997	0.9898	0.9910
	PIC	0.81	0.80	0.80
	Shannon Index	1.8836	1.8668	2.0706
MCW39	**PD**	0.992	0.9904	0.9853
	PIC	0.67	0.79	0.77
	Shannon Index	0.7306	1.7764	1.7625
MCW34	**PD**	0.994	0.9907	0.9861
	PIC	0.78	0.58	0.60
	Shannon Index	1.6754	1.3611	1.5397

DISCUSSION

In the global test for deviation from Hardy-Weinberg equilibrium significant deviation was found in three populations. Deviations from expected values may be due to a variety of causes: inbreeding or out breeding, selection, population substructure, existence of `null alleles' (10). Inbreeding and selection may be ruled out, since neither one of the populations showed only positive or negative deviations nor did any of the loci show a systematic deviation across populations. `Null alleles' have not been described for the microsatellite chosen and no problems were encountered in obtaining PCR fragments at any of the loci.

In studied population's allelic number of the loci varied from 2 to 10. Lowest allelic number was belong to ADL262 (2) in Khazak and highest for ADL225 (10) in Dashtiari. Mean allelic number at different marker loci among three populations were 5.2, 5 and 6.4 for Zabol, Khazak and Dashtiari chicken, respectively. The allelic number range observed in the present investigation is in close agreement with other studies as 17, 18 (2003) (range 5-10 allele in 7 Chinese native chicken), 19 (range 2-7 allele in 12 Chinese native

chicken), 20 (range 3-4 allele in 4 chins native populations) other report among leghorn lines showed 3.6- 4 allelic number (21). The number of alleles at different marker loci is simple indicators of the genetic variability. The results suggest existence of enough genetic variation in the three native chickens for further breeding programs.

The results show that means of heterozygosity was 0.77, 0.79 and 0.52 for Khazak, Dashtiari and Zabol chicken, respectively. This level of genetic diversity for Khazak and Dashtiari is similar to values report in other natives chicken as 20 and 22. Heterozygosity in Zabol chicken is in agreement with researches reported by 19 and 23. Low heterozygosity in Zabol population represents a distinct, well-defined local breed kept and bred as a closed flock. The other populations showed higher heterozygosity. They also represent distinct breeds but were not kept as closed flocks as stringently nor as long as the Zabol chicken. Using the mixture of high diversity microsatellite and low diversity microsatellite will reduce overestimate genetic diversity. Difference between results may be in results to differences in location of sampling, different sample sizes and sources of the microsatellite markers used. Mostly, with increasment of the number of allele, Shannon index and heterozygosity also will increase.

The polymorphism information content (PIC) is another important measure of DNA polymorphism. Besides being a measure of genetic variation, it is also used in the context of genetic mapping. In the study the polymorphism information content (PIC) ranged from 0.50 to 0.81 that related to allelic number.

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