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The Investigation of Genetic Variation at Microsatellite Loci in Mazandaran Native Chickens

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Abstract: Blood samples of Mazandaran native chickens were collected. A total of 90 genomic DNAs were isolated through optimized and modified salting-out procedure. The samples were used in Polymerase Chain Reaction (PCR) with 20 micro satellite markers. Amplified PCR-products with the markers were separated on 8% denaturing polyacrylamide gel. One locus (MCW216) was monomorphic. According to allele frequencies of 20 micro satellite sites, mean heterozygosity (H) and Polymorphism Information Content (PIC) were calculated. The number of alleles varying from 1 to 6 and an estimate of average heterozygosity excluding the monomorphic data was calculated as 0.5872. The average heterozygosity and PIC value calculated from data on polymorphic and monomorphic loci was 0.5579 and 0.4939, respectively. The results of the heterozygosity were consistent with that of PIC. Diversity estimates in this study are lower than the observed frequencies of heterozygotes reported in other species using micro satellite markers. Other parameters for intrapopulation variation and Hardy-Weinberg proportions were also considered. All the loci except MCW222 and MCW165 showed deviations from Hardy-Weinberg equilibrium ($p<0.005$). Some of micro satellite sites were highly polymorphic, so they were effective markers for genetic diversity analysis. These results could provide basic molecular data for the research on the germplasm characteristics of Mazandaran native chickens.

Key words: Micro satellite, heterozygosity, native chicken, polymorphism

Introduction

Biodiversity among domestic animals in developing countries is enormous. However, with the introduction of superior animals breeds with excellent performance, the native animal resources with good adaptability but lower productivity are in great danger (Mirhoseinie *et al*., 2005). According to the study done by FAO up to 30% of Global mammalian and avian Livestock breeds are faced currently at risk of being lost and could not be replaced (Soysal *et al*., 2003). Erosion of genetic diversity in a breed may cause increase in the rate of inbreeding and genetic abnormalities, thereby decrease in animal performance. These will virtually reduce the global gene pool for future development and can be considered as a serious threat for universal food security. Therefore, the urgency and need for conservation of genetic resources in animal biodiversity is clear, particularly for those in developing countries (Mirhoseinie *et al*., 2005).

Poultry products are important sources of high quality protein for human nutrition and knowledge of the chicken genome has the potential generate technologies that will increase efficiency of meat and egg production (Nones *et al*., 2005). The molecular genetic diversity will play an important role in conservation, supervision and utilization of the chicken resources (Lujiang *et al*., 2005). Currently, micro satellites are widely used since they are numerous, randomly distributed in the genome, highly polymorphic and show co-dominance inheritance (Hillel *et al*., 2003).

Mazandaran is an important pole of agriculture and animal husbandry of Iran and approximately have 4000000 native chickens. So, the objective of this study was to assess the genetic diversity of this population for better utilization in breeding programs.

Materials and Methods

Blood samples of 90 Mazandaran native chickens were randomly collected from the wing vein using EDTA as an anti-coagulating agent. Blood samples were stored at-20°C. DNA was extracted from the whole blood using optimized and modified salting-out method (Miller *et al*., 1988). DNA was quantified spectrophotometrically and concentration was adjusted to 50 ng μL^{-1}. Genomic DNA (50ng) was amplified with 1 unit Taq polymerase, 4.5-5.6mM $mgcl_2$, 200 μM dNTPs and 0.25 μM of each primer in a total volume of 15 μL.

Twenty micro satellites were used in this study are listed in Table 1. this loci previously used by Crooijmans *et al*., 1996; Crooijmans *et al*., 1997; Hillel *et al*., 2003; Olowofeso *et al*., 2005; Thi Kim Cuc *et al*., 2006; Gibbs *et al*., 1997; Ya-BO *et al*., 2006. The loci were chosen on the basis of their location in several chromosomes. The reaction mixture was subjected to an initial 5 min denaturation at 94°C, followed by 28-35 cycles of denaturation at 94°C for 30s, annealing at 55-65°C (depending on locus) for 45″s, extension at 72°C for 1′:30″ and a final extension step at 72°C for 2 min. after the addition of 10 μL of formamide solution, 10 μL of

Table 1: Sequence, reported and observed alleles of primers

Observed allele range (bp)	Primer sequences (5-3´)	Reported allele rang (bp)	Locus
165-194	TATTGGCTCTAGGAACTGTCGAAATGAAGGTAAGACTAGC	173-190*	MCW0014
255-310	ATCCCAGTGTCGAGTATCCGATGAGATTTACTGGAGCCTGCC	290-311**	MCW0183
100-120	GCACTACTGTGTGCTGCAGTTTGAGATGTAGTTGCCACATTCCGAC	114-124***	ADL0278
167-192	*GCACTACTGTGTGCTGCAGTTTGAGATGTAGTTGCCACATTCCGAC*	*175-184***	*MCW0067*
206-220	GTTGTTCAAAAGAAGATGCATGTTGCATTAACTGGGCACTTTC	216-225**	MCW0248
170-187	TCTTCTTTGACATGAATTGGCAGCAAGGAAGATTTTGTACAAAATC	179-187***	MCW0020
217-253	TGCACGCACTTACATACTTAGAGATGTCCTTCCAATTACATTCATGGG	214-242***	MCW0034
218-228	GCAGTTACATTGAAATGATTCCTTCTCAAAACACCTAGAAGAC	221-225**	MCW0222
172-225	GATCTCACCAGTATGAGCTGCTCTCACACTGTAACACAGTGC	253-285**	LEI0094
145	GGGTTTTACAGGATGGGACGAGTTTCACTCCCAGGGCTCG	141-147***	MCW0216
194-225	TAGCACAACTCAAGCTGTGAGAGACTTGCACAGCTGTGTACC	190-230***	MCW0104
242-296	TGGACCTCATCAGTCTGACAGAATGTTCTCATAGAGTTCCTGC	260-290**	MCW0330
118-122	CAGACATGCATGCCCAGATGAGATCCAGTCCTGCAGGCTGC	114-118***	MCW0165
86-100	CCACTAGAAAAGAACATCCTCGGCTGATGTAAGAAGGGATGA	76-98***	MCW0123
104-112	CTCCACCCCTCTCAGAACTACAACTTCCCATCTACCTACT	104-116***	ADL0268
247-260	CTCCTGCCCTTAGCTACGCATATCCCCTGGCTGGGAGTTT	254-267**	LEI0166
98-112	ATCACTACAGAACACCCTCTCTATGTATGCACGCAGATATCC	94-107**	MCW0295
118-125	GTTGCTGAGAGCCTGGTGCAGCCTGTATGTGGAATTACTTCTC	114-143***	MCW0081
112-133	GGCTTAAGCTGACCCATTATATCTCAAATGTAATGCGTGC	124-132***	ADL0112
159-170	GCACTCGAGAAAACTTCCTGCGATTGCTTCAGCAAGCATGGGAGGA	158-176***	MCW0069

*Vanhala *et al.* (1998), **Crooijmans *et al.* (1997), ***Thi Kim Cuc *et al.* (2006)

amplification products were loaded on to 8% denaturing polyacrylamide gels. To visualize the PCR product, gels were stained using silver staining (Bassam and Caetano-Anolles, 1993). The stained gels were scanned and genotypes were scored.

POPGENE software was used to estimate the observed and expected heterozygosity and effective number of alleles (Yeh *et al.*, 1999). Average expected theoretical heterozygosity from Hardy-Weinberg assumptions was calculated using the formula (Hedrick, 1999):

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

Where, p_i is the ith allele frequency.

Effective number of alleles (n_e) was calculated using the formula (Hedrick, 1999):

$$n_e = \frac{1}{\sum_{i=1}^{n} P_i^2}$$

PIC was estimated, using HET software package (Ott, 1988). Polymorphic Information Content (PIC) was calculated using the formula (Mirhoseinie *et al.*, 2005).

$$PIC = 1 - \left(\sum_{i=1}^{k} P_i^2 \right) - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2P_i^2 P_j^2$$

Where, p_i and p_j are frequencies of corresponding alleles.

Results and Discussion

PCR amplification was carried out using 20 micro satellite primers listed in Table 1. Genetic parameters are shown in Table 2. Population was monomorphic at MCW216 locus. The size of the amplified bands ranged between 86 bp (MCW123) to 310 bp (MCW183). Chi square (x^2) test was used to evaluate Hardy-Weinberg equilibrium. All loci excluding MCW222 and MCW165 were found to be deviating from Hardy-Weinberg equilibrium ($p<0.005$).

The minimum (1 allele) and maximum number of alleles (6 alleles) were observed at MCW216 locus and MCW34, respectively. The average allele number for micro satellite markers was 3.45±1.3563.The minimum (1) and maximum (3.74) of effective number of alleles were obtained at MCW216 and MCW123, respectively. The mean effective number of alleles was 2.59±0.8550. The observed heterozygosity of MCW165 was lowest and that of MCW123 highest. The expected heterozygosity of micro satellite markers ranged from 0.2472 to 0.7328 that belonged to MCW165 and MCW123, respectively.

An estimate of average heterozygosity excluding the monomorphic data was calculated as 0.5872. The average heterozygosity and PIC value calculated from data on polymorphic and monomorphic loci was 0.5579 and 0.4939, respectively.

17 loci were deviating from HWE. Vanhala *et al.* (1998) in study on the eight chicken lines found that three loci (from 9 loci) deviated from the HWE. Association of loci with some genes that are of some economics importance, presence of null alleles and the gel condition are their reasons. In this study, no

Table 2: Genetic parameters in Mazandaran native chicken with 20 micro satellite loci

Locus	n[a]	n_e[b]	H_o[c]	H_e[d]	PIC[e]
ADL268	5	3.64	0.9667	0.7254	0.6805
ADL278	2	1.8	0.6667	0.4444	0.3457
MCW295	5	3.08	0.8444	0.6755	0.6217
MCW248	3	1.83	0.5333	0.4543	0.3926
MCW20	3	2.68	0.8	0.6277	0.5571
MCW123	4	3.74	1	0.7328	0.6842
LEI166	2	1.91	0.7889	0.4777	0.3636
MCW330	4	3.67	0.6333	0.7277	0.6777
MCW222	2	1.44	0.3778	0.3064	0.2595
MCW67	2	1.97	0.8667	0.4938	0.3719
MCW138	3	2.65	0.8	0.6228	0.5516
MCW104	5	3.49	0.6222	0.7138	0.6650
MCW34	6	3.15	0.9889	0.6835	0.6269
ADL112	4	3.35	0.9778	0.7018	0.6476
LEI94	4	2.99	1	0.6665	0.6041
MCW14	4	3.08	0.8444	0.6762	0.6141
MCW69	5	2.97	0.6333	0.664	0.5995
MCW165	2	1.32	0.2889	0.2472	0.2166
MCW81	3	2.06	0.9889	0.5159	03990
MCW216	1	1	0.000	0.000	0.000
Mean	3.45	2.5951	0.7311	0.5579	0.4939
S.E	1.3563	0.8550	0.2695	0.1940	

a: number of observed alleles, b: number of effective alleles,
c: bserved heterozygosity, d: expected heterozygosity,
e: polymorphism information content. S.E. = Standard error

homozygous null individuals were found. Scoring bias may be possible for a few loci but not for all loci. So, it could be caused by selection, migration, the finite size of population, phenotypic assortative mating and high mutation rate of micro satellite.

In total, 69 alleles were found at 20 loci in this population. Barker (1994) suggested that micro satellite loci used in studies of genetic distance should have more than four alleles in order to reduce the standard errors of distance estimates, thus some micro satellite markers in this study were suitable for genetic diversity. Differences in allele size can be interpreted as population specific alleles or scoring biase.

It is considered that, loci are highly polymorphic where PIC>0.5 (Ya-Bo *et al*., 2006) some loci studied were highly polymorphic and with the highest value 0.684 (MCW123). The highest heterozygosity (0.7328) was also belonging to MCW123. It was found that a comparing heterozygosity with PIC, all PIC values were less than their related heterozygosity and the results of the heterozygosity were consistent with that of PIC. This result was agreed with other studies (Yj *et al*., 2005; Mirhoseinie *et al*., 2005).

The level of genetic diversity estimated in this study were higher than other values reported for different chicken populations using micro satellite markers, Hillel *et al*. (2003) for some European chicken populations and Dai *et al*. (2006) for Chinese chicken populations. It was also lower than some reports. The variation in results could be adduced to differences in location, sample sizes, experimental chicken and sources of the microsatellite markers used. Diversity estimates in this study as the same as other studies are lower than the observed frequencies of heterozygotes reported in other species using micro satellite markers. For instance, in human populations the average heterozygote frequency ranges between 0.7 and 0.8, in cattle 0.6, in pigs 0.68 and in fish 0.86. Although such comparisons are difficult to interpret, the lower variability in chickens calls attention to the importance of conserving the chicken gene pool (Hillel *et al*., 2003).

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