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Genetic Variation and Relationships of Korean Native Chickens and Foreign Breeds Using 15 Microsatellite Markers

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ABSTRACT : The purpose of this study was to assess the genetic variation and establish the relationship amongst breeds and strains using 15 chicken specific microsatellite markers. A total of 285 unrelated DNA samples from four Korean native chicken strains (Black strain of Korean native chicken; KL, Red Brown strain of Korean native chicken; KR, Ogol strain of Korean native chicken; KS and Yellow Brown strain of Korean native chicken; KY) and three introduced chicken breeds (F strain of White Leghorn; LF, K strain of White Leghorn; LK, Rhode Island Red; RC and Cornish; CN) were genotyped to estimate within and between breed genetic diversity indices. All the loci analyzed in 15 microsatellite markers showed a polymorphic pattern and the number of alleles ranged from 5 to 14. The polymorphism information content (PIC) of UMA1019 was the highest (0.872) and that of ADL0234 was the lowest (0.562). The expected total heterozygosity (He) within breed and mean number of observed alleles ranged from 0.540 (LF) to 0.689 (KY), and from 3.47 (LK) to 6.07 (KR), respectively. The genetic variation of KR and KY were the highest and the lowest within Korean native strains, respectively. The genetic distance results showed that Korean native chicken strains were separated with the three introduced chicken breeds clustered into another group. The lowest distance (0.149) was observed between the KR and KL breeds and the highest distance (0.855) between the KR and LK breeds. The microsatellite polymorphism data were shown to be useful for assessing the genetic relationship between Korean native strains and other foreign breeds. (**Key Words :** Korean Native Chicken (KNC), Microsatellite Loci, Genetic Relationship, Heterozygosity)

INTRODUCTION

The Native chicken breeds are becoming endangered or even extinct because of their poor commercial performances. Korean Native Chicken (KNC) also reduced in number drastically since early 20th century with the introduction of new breeds. The chicken industry was thriving on new imported synthetic breeds, while only few farms in remote areas raised these native chickens. In the late 1970s, the National Livestock Research Institute started a conservation project and collected chickens from remote farms on the basis of phenotypic and feather colors. Through the multiplication program, the Korean native chicken population increased. Korean farmers reared KNC both for meat and eggs since it is a dual purpose breed. KNC are less fatty and higher protein content compared to foreign breeds. Therefore it is very popular to domestic consumers.

In the process of evaluating genetic diversity to develop conservation measures in chicken, it is of special interest to assess genetic variation between different chicken breeds by utilizing modern molecular tools (Groene et al., 2000; Osman et al., 2006). Monolocus microsatellites have been shown to be suitable markers for this purpose and may resolve genetic relationships between closely related population (Tautz, 1989). Microsatellite loci are widely dispersed along and among chromosomes and each locus is characterized by a known DNA sequence. They are typically composed of between two to four nucleotides such as (CT)n or (GATA)n where n lies between 5 and 50 (Dewoody and Avise, 2000). Many kinds of microsatellites are informative due to their high polymorphism. Microsatellite markers are useful in paternity testing, identification testing and breed assignment analysis (Sirchia et al., 1996; Bowling et al., 1997; Yoon et al., 2005; Fan et

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Table 1. 15 microsatellite primer sequences and part of PCR conditions in this study

Loci	Forward primer (5'-3')	Reverse primer (5'-3')	Tm (°C)
ADL0019	TGCAACTAAGTTGTGGACTG	TCTGCTGGGATTATGTGTCA	50
MCW0106	GGCAACTAAGTTGTGGACTG	GCAGCATTCAGTGGGATAAT	50
GCT0006	ATTTCCTATTCCCCTCTC	CCAGAAAACATCACCAAC	50
MCW0145	ACTTTATTCTCCAAATTTGGCT	AAACACAATGGCAACGGAAC	55
UMA1019	ACACTGGCAGGCGTGTTAG	GCTTGAGGACAGGGGTCAGG	55
ADL0020	TAGATAAAATCCTTCCCTT	GCAGTGAAAAGAAAAGAAT	55
ADL0234	CTGGACGCGTGAAAAAGTTC	CCCTGGGGCTCCCTCAGCAC	55
LEI0169	TTGCTTGTTTGCTGCCTTTTAG	ACAGTGTAGCATGGACAACAG	55
MCW0023	TAAAGCTGAGCCTGGGGAACCTAA	ATCCATTTACTGTGAAACAG	55
UMA1125	CCAGCATGTGATTCCCAAGT	AGTGTTTCCAGGGGCAAGGA	55
ADL158	TGGCATGGTTGAGGAATACA	TAGGTGCTGCACTGGAAATC	60
ADL181	CAATCTTTTGTGGGGTATGG	CCAGTGAAATTCATCCTTTT	50
ADL176	TTGTGGATTCTGGTGGTAGC	TTCTTCCGTAACACTCGTCA	60
ADL267	AAACCTCGATCAGGAAGCAT	GTTATTGAAAGCCCCACCAC	60
ADL172	CTATGGAATAAAATGGAA AT	CCCTACAACAAAGAGCAGTG	50

Tm: Annealing temperature.

al., 2006). Also microsatellite markers have proven in assessing genetic variation and diversity in livestock (Buchanan et al., 1994; MacHugh et al., 1994; Martinez et al., 2000; Pandey et al., 2002). To analysis of genetic diversity polymorphism and relationships in native breeds must study for preservation and improvement of genetic resources. However, very little information is available concerning the genetic diversity of KNC breeds.

The present study was conducted to characterize genetic diversity and relationship among chicken breeds including KNC breeds based on allelic frequencies for 15 microsatellite markers.

MATERIAL AND METHODS

Animals and DNA extraction

A total of 285 chickens were used in this study. Four native Korean breeds (KL: Black strain of Korean native chicken 30, KB: Red Brown strain of Korean native chicken 45, KS: Ogol strain of Korean native chicken 40, KY: Yellow Brown strain of Korean native chicken 30) and four foreign breeds were assessed (CN; Cornish 40, LF; Leghorn F 30, LK; Leghorn K 30, RC; Rhode Island Red 40). The blood samples were collected from the ulnar vein. Genomic DNA samples were extracted from blood by some modification of the method used by Miller et al. (1988).

Microsatellite loci

The 15 microsatellite markers chosen for analysis were : ADL0019, MCW0106, GCT0006, MCW0145, UMA1019, ADL0020, ADL0234, LEI0169, MCW0023, UMA1125, ADL158, ADL181, ADL176, ADL267, ADL172.

PCR and Genotyping

PCR was conducted with a final volume of 10 μl, including 1 μl of 10 X reaction buffer (10 mM Tirs, pH 8.3, 50 mM KCL, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 0.7 μl dNTP Mix (2.5 mM), 10 pM of each primer, 20 ng of genomic DNA, and 0.5U of Taq polymerase. Amplification of PCR products was carried out using a standard PCR program with 5 min denaturation at 94°C, 30 cycles for 30 sec at 94°C, 30 sec annealing at 50-60°C, 1 min extension at 72°C, and final extension for 10 min at 72°C. PCR product 1 μl were mixed with 0.5 μl GS -400 TAMRA size standard (DNA fragments of known size labeled with ABI PRISM dye N, N, N1-tetra-methyl-6-carboxy-rhodamine (TAMRA) (Perkim-Elmer, USA), 8 μl loading formamid solution. The samples were denaturized by heating at 90°C for 5-min followed by cooling on ice. Analyses of PCR products were performed by ABI 3100 Genetic Analyzer (Applied Biosystems, USA). Relative ratios of the detected virus sequences were determined by comparison of peak area values fork each of the detected fragments.

Statistical analysis

MS toolkit s/w (Park, 2000) was used to estimate the eterozygosity frequency and marker allele frequency. The heterozygosity was calculated according to Nei et al. (1978). The number of alleles per locus were estimated by direct counting from observed genotype. Polymorphic Information Content (PIC) and expected allelic diversity (Div) was calculated by using the method described by Nei et al. (1972, 1978). Genetic variability estimates of average observed heterozygosity, expected total heterozygosity (H_t), expected within population heterozygosity (H_s), coefficient of gene differentiation (G_{st}), total and mean number of alleles per population were calculated using DISPAN (Ota, 1993).

Phylogenetic analysis

The standard genetic distances (Nei et al., 1972) were

Table 2. Characterization of the 15 microsatellite loci analyzed in 8 breeds and strains

Marker name	No. of Alleles	Size range (bp)	PIC	H_o	Div
ADL0019	8	98-116	0.740	0.620	0.772
MCW0106	7	117-135	0.703	0.637	0.763
GCT0006	6	189-199	0.613	0.538	0.675
MCW0145	14	178-214	0.794	0.681	0.817
UMA1019	14	138-166	0.872	0.677	0.883
ADL0020	7	93-108	0.694	0.580	0.785
ADL0234	5	150-164	0.562	0.563	0.653
LEI0169	8	230-250	0.657	0.506	0.707
MCW0023	9	151-181	0.648	0.657	0.698
UMA1125	7	138-166	0.724	0.616	0.761
ADL158	11	183-207	0.616	0.496	0.678
ADL181	6	174-184	0.657	0.506	0.733
ADL176	8	173-205	0.718	0.531	0.790
ADL267	10	97-117	0.685	0.551	0.712
ADL172	11	130-158	0.809	0.602	0.846
Average	8.733		0.699	0.584	0.751

PIC: Polymorphism information content, H_o: Observed heterozygosity, Div: Expected allelic diversity.

Table 3. Characterization of 15 microsatellite loci analyzed with 8 population

Marker name	G_{st}	H_t	H_s
ADL0019	0.208	0.764	0.605
MCW0106	0.119	0.750	0.661
GCT0006	0.204	0.681	0.542
MCW0145	0.136	0.824	0.712
UMA1019	0.128	0.880	0.768
ADL0020	0.228	0.781	0.603
ADL0234	0.163	0.640	0.536
LEI0169	0.217	0.716	0.560
MCW0023	0.164	0.690	0.577
UMA1125	0.213	0.768	0.604
ADL158	0.191	0.709	0.573
ADL181	0.169	0.723	0.601
ADL176	0.192	0.793	0.641
ADL267	0.251	0.753	0.565
ADL172	0.133	0.847	0.735
All loci	0.180	0.755	0.619

G_{st}: Gene differentiation, H_t: Expected total heterozygosity, H_s: Expected within population heterozygosity.

calculated between all pairs of breeds, and the phylogenetic tree was constructed based on the unweighted pair-group method with arithmetic mean (UPGMA) method (Sneath and Sokal, 1973). The reliability of the tree obtained was examined by a bootstrap test with 1,000 replicate resampling of loci with replacement. These procedures were conducted by using the DISPAN program, and the tree was visualized on the TREEVIEW program.

RESULTS AND DISCUSSION

Polymorphism of microsatellite

Number of alleles, size range of alleles, polymorphism information content (PIC), observed heterozygosity (H_o) and expected allelic diversity (Div) are given in Table 2. A total of 132 alleles were observed at the 15 loci in 285 individuals from 8 chicken breeds. All the loci were polymorphic and the number of alleles ranged from 5 (ADL0234) to 14 (MCW0145 and UMA1019) with an average value of 8.733. The highest PIC value was UMA1019 (0.872) and the lowest was ADL0234 (0.562) with an average value of 0.699. The calculated observed heterozygosities for the whole population were between 0.496 and 0.681 (the average value was 0.584). The highest H_o value was MCW0145 (0.681) and the lowest was ADL158 (0.496). The expected allelic diversity (Div) ranged from 0.883 to 0.653 (the average value was 0.751). The UMA1019 was calculated highest PIC and Div values. The other side, the ADL0234 was calculated lowest PIC and Div values. Measures of genetic variability were in Table 3. The coefficient of gene differentiation (G_{st}) and expected total heterozygosity (H_t) ranged 0.119-0.251 (the average value was 0.180) and 0.709-0.880 (the average value was 0.755) in 8 populations, respectively.

Genetic diversity and variation within population

Table 4 showed that means of expected heterozygosity (H_e), observed heterozygosity (H_o) and PIC estimated for 8 breeds. There means in 8 breeds were obtained by calculating the gene frequency of different genes. KR had highest mean number of allele (6.07), H_o (0.662) and PIC (0.630) values. PIC values of CN, KL, KR, KS, KY, and RC were high, but LF, LK were lower than the other breeds. LF and LK showed lower expected heterozygosity (0.540 and 0.587, respectively) and mean number of allele (3.47 and 3.40, respectively) than the other breeds. The other side, Korean native strains showed comparatively high means (Mean no. of allele, H_e, H_o and PIC). The genetic variation of KR and KY were the highest and the lowest within Korean native strains, respectively.

Table 4. Mean number of alleles, expected heterozygosity (H_e), observed heterozygosity (H_o) and polymorphism information content (PIC) of microsatellite loci for each breeds

Breeds	Pop. size	Mean no. of allele	H_e	H_o	PIC
CN	40	5.93	0.627	0.592	0.532
KL	30	5.07	0.621	0.607	0.546
KR	45	6.07	0.679	0.662	0.630
KS	40	5.67	0.679	0.570	0.616
KY	30	5.33	0.689	0.557	0.585
LF	30	3.47	0.540	0.577	0.454
LK	30	3.40	0.587	0.583	0.493
RC	40	4.73	0.622	0.560	0.561
Pooled sample	285	4.95	0.630	0.588	0.552

PIC: Polymorphism information content.
H_e: Expected heterozygosity, H_o: Observed heterozygosity.
CN: Cornish, KL: Black strain of Korean native chicken (KNC).
KR: Red Brown strain of KNC, KS: Ogol strain of KNC.
KY: Yellow Brown strain of KNC, LF: Leghorn F, LK: Leghorn K.
RC: Rhode Island Red.

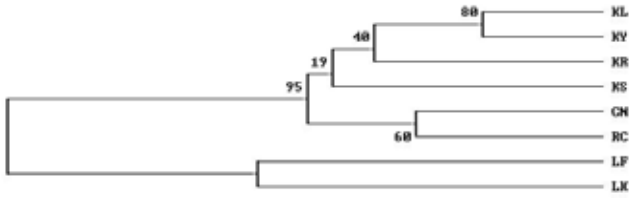

Figure 1. UPGMA tree showing the genetic relationships among four breeds of Korean native chickens and four foreign breeds (Leghorn F, Leghorn K, Rhode Island Red, Cornish) using standard genetic distances calculated from 15 microsatellites. The number at the node indicate bootstrap values in percentage (1,000 replicates). CN: cornish, KL: Black strain of Korean native chicken (KNC), KR: Red Brown strain of KNC, KS: Ogol strain of KNC, KY: Yellow Brown strain of KNC, LF: Leghorn F, LK: Leghorn K, RC: Rhode Island Red.

Genetic relationship analysis between populations

Matrix of genetic distances between every pair of breeds was calculated from the allele frequencies at the 15 microsatellites and is presented in Table 5. The dendrogram drawn from the genetic distance matrix among 8 breeds is shown in Figure 1. The average genetic distance among all breeds was 0.5±0.22. The lowest distance (0.149) was observed between the KY and KL breeds, and the highest distance (0.855) between the KR and LK breeds. The average genetic distance among Korean native strain (KL, KR, KS and KY) population was 0.282. The average genetic distance between CN and Korean native chicken (KNC) was 0.380, and between RC and KNC was 0.345, and between LF and KNC was 0.683, and between LK and KNC was 0.712. CN and RC were near genetic distance comparatively with KNC population. But LF and LK were far away genetic distance comparatively with KNC population. The results showed that KNC strains were separated with the three introduced chicken breeds clustered into another group. KL, KR, KS and KY were grouped together although. CN and RC belonged to another group. Like wise, LF and LK were grouped together.

Microsatellite DNA can be a useful tool in genetic studies such as parentage determination, population studies, linkage analysis and genom mapping. In the present study was using 15 microsatellite markers to understand genetic diversity of KNC strains. KNC strains and foreign breeds were analyzed to investigate a phylogenetic distribution relationship among the populations. A total of 132 alleles were observed at the 15 loci in 285 individuals from 8 chicken breeds. All the loci were polymorphic and the number of alleles ranged from 5 to 14 with an average value of 8.733. Averages of genetic variability showed that observed heterozygosity was 0.584, PIC was 0.699, coefficient of gene differentiation (Gst) was 0.180 and expected total heterozygosity (Ht) was 0.755. Korean native strains showed comparatively high means within no. of alleles, He, Ho and PIC. The genetic variation of KR and KY were the highest and the lowest within Korean native strains, respectively. The results showed that KNC strains were separated with the three introduced chicken breeds clustered into another group. In 1970's, genetic sources of Korean Native Chicken were reorganized to standardize the characteristics of KNC. Around those days, KNC were categorized into 4 strains in terms of appearance color patterns. However, up until 1970's, they had been crossed

Table 5. Matrix of standard genetic distances (below diagonal) and standard error (above diagonal) estimated between 8 chicken breeds based on 15 microsatellites

	CN	KL	KR	KS	KY	LF	LK	RC
CN	0	0.090	0.070	0.087	0.095	0.196	0.154	0.068
KL	0.360	0	0.102	0.067	0.035	0.156	0.166	0.083
KR	0.269	0.322	0	0.080	0.080	0.148	0.164	0.074
KS	0.464	0.295	0.409	0	0.062	0.146	0.159	0.097
KY	0.428	0.149	0.236	0.283	0	0.146	0.127	0.066
LF	0.834	0.590	0.787	0.754	0.603	0	0.115	0.153
LK	0.804	0.674	0.855	0.617	0.704	0.423	0	0.173
RC	0.225	0.269	0.331	0.434	0.349	0.731	0.812	0

CN: Cornish, KL: Black strain of Korean native chicken (KNC). KR: Red Brown strain of KNC, KS: Ogol strain of KNC.
KY: Yellow Brown strain of KNC, LF: Leghorn F, LK: Leghorn K. RC: Rhode Island Red.

among them and thus, the 4 strains were not well kept separately from each other. Since late 1970's, the 4 strains have been re-bred within strains to recover the 4 pure strains throughout intensive and well-controlled selection programs. Nevertheless, there exist very similar genetics characteristics among the 4 strains because they were once crossbred and selected only for the appearance colors. Therefore, genetic relationship, which were based the microsatellite markers, among the 4 strains were found much similarities. Result, obtained in these analysis indicated that microsatellite may provide data substantial equivalent to genetic background information. The results will be useful to make decisions regarding preservation and further use development of the native breeds.

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