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Discrimination of Korean Native Chicken Lines Using Fifteen Selected Microsatellite Markers

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ABSTRACT: In order to evaluate the genetic diversity and discrimination among five Korean native chicken lines, a total of 86 individuals were genotyped using 150 microsatellite (MS) markers, and 15 highly polymorphic MS markers were selected. Based on the highest value of the number of alleles, the expected heterozygosity (He) and polymorphic information content (PIC) for the selected markers ranged from 6 to 12, 0.466 to 0.852, 0.709 to 0.882 and 0.648 to 0.865, respectively. Using these markers, the calculated genetic distance (*Fst*), the heterozygote deficit among chicken lines (*Fit*) and the heterozygote deficit within chicken line (*Fis*) values ranged from 0.0309 to 0.2473, 0.0013 to 0.4513 and -0.1002 to 0.271, respectively. The expected probability of identity values in random individuals (PI), random half-sib ($PI_{half\text{-}sibs}$) and random sibs (PI_{sibs}) were estimated at 7.98×10^{-29}, 2.88×10^{-20} and 1.25×10^{-08}, respectively, indicating that these markers can be used for traceability systems in Korean native chickens. The unrooted phylogenetic neighbor-joining (NJ) tree was constructed using 15 MS markers that clearly differentiated among the five native chicken lines. Also, the structure was estimated by the individual clustering with the K value of 5. The selected 15 MS markers were found to be useful for the conservation, breeding plan, and traceability system in Korean native chickens. (**Key Words:** Discrimination, Diversity, Microsatellite, Korean Native Chicken, Traceability)

INTRODUCTION

The Korean native chicken has been documented since approximately 2,000 years ago. Due to their poor commercial performance, Korean native chicken breeds almost became extinct. For this reason, Korean native chicken conservation strategies have been launched by the Korean government in 1994. Based on the 2 decades of the conservation project's duration, five native chicken breeds with nine lines have been developed. In Korea, approximately 90% of chicken meat consumption is based on imported breeds. Recently, the poultry meat production has steadily increased, accounting for up to 20% of the total meat consumption in Korea (MIFAFF, 2009). Nowadays, many Korean consumers have a higher preference for native chicken meat than before, even though they may be 2 to 3 times as expensive as broilers.

Evaluation of genetic diversity for local breeds is becoming more challenging, and large efforts have been concentrated on maintaining minimum number of animals for each native species (FAO, 2007). There are extensive advantages of microsatellite (MS) markers because MS markers are abundant repeats of one to six bases, exhibit co-dominant inheritance, and are highly polymorphic and dispersed throughout the genome (Cheng and Crittenden, 1994; Kaya and Yildiz, 2008). In the Ark Database, the documented chicken MS markers are 2,483 markers, of which 435 are unmapped (Jacobsson et al., 2004). Until now, MS markers are the most widely used for the improvement of genetic selection management, parentage studies, evolutionary analysis, genetic traceability systems and QTL mapping (Blott et al., 1999; Dalvit et al., 2007; Almasy and Blangero, 2009). Previously, twenty two MS markers were used to assess chicken domestication in 52 populations. The results from identified alleles and the

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amount of genetic variation supported the hypothesis that the red jungle fowl was the ancient progenitor (Hillel et al., 2003). Therefore, MS markers are more suitable for the study of genetic diversity, correlation studies, and for identifying population structure among the chicken populations (Kong et al., 2006; Muchadeyi et al., 2007; Mwacharo et al., 2007; Tadano et al., 2007a; Tadano et al., 2007b; Berthouly et al., 2008; Bodzsar et al., 2009; Ding et al., 2010). On the other hand, the single nucleotide polymorphism (SNP) markers in MC1R gene were not sufficient for the discrimination of these Korean native chicken lines (data has not shown). Also, the phylogenetic relationships between Korean native chicken and other breeds have been investigated using D-loop sequence variations in mtDNA and attempts for discrimination of Korean native chicken lines were performed using mtDNA and LEI0258 marker (Hoque et al., 2009; Hoque et al., 2011).

In our studies, 15 markers have been selected from the 150 MS markers in the Ark database in order to investigate Korean native chicken lines to identify their genetic relationships. Also, these markers were used for the calculation of discrimination probabilities, which can be used for chicken traceability systems. Also, these results can be used in further breeding and conservation strategies for Korean native chicken.

MATERIALS AND METHODS

Sample collection and DNA extraction

Five Korean native chicken lines were collected from the National Institute of Animal Science (NIAS) in Korea. These lines were basically classified according to their feather colors, which were white (KNC_W), black (KNC_B), red-brown (KNC_R), yellow-brown (KNC_Y) and gray (KNC_G) lines. A total of 86 individuals were used for DNA extraction from blood samples collected from wing veins in tubes containing EDTA. Samples were stored at -20°C and genomic DNAs were extracted using a manual extraction method (Miller et al., 1988).

PCR amplification and genotyping

Initially, 150 MS markers were selected from the Ark Database (http://www.thearkdb.org/arkdb/) and were genotyped (Table 1). The primers used for genotyping were labeled with four fluorescence dyes (FAM, NED, VIC, PET) in forward primers. For the discrimination analysis of chicken lines, 15 highly polymorphic microsatellite markers were selected based on the number of alleles, expected heterozygosity (He) and polymorphic information content (PIC) values (Table 2). PCR was performed in an initial denaturation at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C and a final extension at 72°C for 10 min using My-Genie96 Thermal Cycler (Bioneer, Korea). The PCR products were initially electrophoresed on 4% agarose gel with ethidium bromide, and DNA bands were visualized under ultraviolet light. For genotyping, the final genotyping reactions were based on 1 μl of 20X diluted PCR products, 10 μl of Hi-Di™ formamide (Applied Biosystems, USA) and 0.1 μl of GeneScan™-500LIZ™ size standard (Appilied Biosystems, USA) in a total volume of 11.1 μl. The microsatellite genotyping was performed using a Genetic Analyzer 3130xl (Applied Biosystems, USA) and the genotyping results were obtained using Genemapper (ver. 3.0, Applied Biosystems, USA).

Statistical analysis

The number of alleles, expected heterozygosity (He), observed heterozygosity (Ho) and polymorphic information content (PIC) and *F*-statistics were calculated for the selected 15 MS markers using the Cervus (ver 3.0) program (Marshall et al., 1998). Expected heterozygosity was derived from an unbiased formula (Nei, 1987) using allele frequencies assuming Hardy-Weinberg equilibrium which is a useful measure of informativeness of a locus. The polymorphism information content (PIC) is a closely related diversity measure which is estimated as:

$$\overline{PIC_l} = 1 - \sum_{u=1}^{k} \tilde{p}_{lu}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_{lu}^2 \tilde{p}_{lv}^2$$

Here, l denotes the lth locus and P_{lu} and P_{lv} are the sample frequencies of a series of alleles A_u and A_v at the lth locus (Botstein et al., 1980). *F*-statistics describe the amount of inbreeding-like effects within subpopulations (F_{st}), among subpopulations (F_{is}), and within the entire population (F_{it}) (Wright, 1965).

The expected probability of identity values among genotypes of random individuals (PI), random half sibs ($PI_{half\text{-}sibs}$) and random sibs (PI_{sibs}) were calculated using API-CALC (ver 1.0) (Ayres and Overall, 2004). This formula is only used for pairs of unrelated individuals such as relatives share genes, and consequently additional loci are likely to be required in order to adequately determine whether two profiles are from distinct individuals as follows:

$$PI_{ave} = \sum_i P_i^4 + \sum_i \sum_{j>i} (2 p_i p_j)_2$$

Where, p_i and p_j are population allele proportions.

Also, genetic distance values were calculated among five native chicken lines using PowerMarker (Ver 3.25)

(Liu and Muse, 2005). A phylogenetic tree was also constructed by Neighbor-Joining tree method (Nei, 1983) method embedded in PowerMarker software package. Finally, in Structure (Ver 2.3.3) program, we assumed five populations (*i.e.,* K = 5) in these chicken lines to get the estimates of proportion of individual's ancestry from those population (Pritchard et al., 2000).

RESULTS AND DISCUSSION

Survival genetic diversity and differentiation

The highest values in the number of alleles, expected

Table 1. The investigated 150 MS markers for Korean native chicken lines. The numbers of alleles are indicated, and the selected 15 MS markers are in bold

Marker	Chr.	Map position (cM)	No. of allele	Allele Size (bp)	Marker	Chr.	Map position (cM)	No. of allele	Allele size(bp)	Marker	Chr.	Map position (cM)	No. of allele	Allele size (bp)
MCW0248	1	21	3	215-223	MCW0016	3	247	5	134-146	**GCT0016**	**9**	**41**	**8**	**108-154**
ADL0160	1	33	2	113-133	GCT0053	3	263	5	128-154	ADL0191	9	44	6	128-150
LEI0194	1	81	7	130-174	ADL0237	3	275	8	133-153	**ADL0021**	**9**	**53**	**8**	**166-188**
MCW0111	1	118	5	98-106	MCW0040	3	282	8	129-147	**ADL0259**	**9**	**122**	**9**	**106-146**
ADL0188	1	133	6	145-165	LEI0166	3	300	3	346-356	MCW0134	9	132	7	260-284
MCW0297	1	162	7	288-304	MCW0037	3	317	3	152-156	MCW0228	10	0	6	221-239
LEI0146	1	169	5	248-268	ADL0143	4	0	4	152-166	MCW0067	10	59	3	176-182
MCW0106	1	94	3	125-131	ADL0255	4	3	4	97-109	ADL0158	10	101	4	188-204
MCW0101	1	248	4	274-280	ADL0317	4	12	9	174-216	ADL0112	10	120	3	127-133
ADL0268	1	288	5	105-117	ADL0203	4	35	7	168-194	MCW0097	11	18	3	267-273
LEI0108	1	300	10	256-310	MCW0295	4	75	5	88-100	ADL0123	11	22	3	106-138
LEI0169	1	400	5	232-248	ADL0241	4	80	7	201-215	MCW0332	12	90	2	196-200
LEI0107	**1**	**424**	**8**	**206-240**	ADL0246	4	112	9	146-164	ADL0147	13	32	4	211-217
MCW0145	**1**	**455**	**7**	**182-210**	ADL0194	4	118	4	198-214	**LEI0251**	**13**	**47**	**12**	**98-132**
MCW0020	1	460	4	183-189	ROS0024	4	148	6	312-328	MCW0216	13	47	4	136-146
LEI0134	1	527	6	291-311	LEI0094	4	153	9	246-280	ADL0310	13	51	10	132-158
MCW0107	1	565	4	110-118	MCW0098	4	217	2	260-262	ROS0083	13	55	7	109-129
LEI0234	2	50	10	217-315	LEI0085	4	231	5	245-259	MCW0322	13	67	3	252-268
MCW0131	2	102	6	196-216	MCW0263	5	28	4	227-249	**MCW0104**	**13**	**74**	**11**	**188-226**
MCW0206	2	104	5	226-240	MCW0193	5	50	9	298-318	ADL0200	14	16	7	112-138
ADL0176	2	116	5	184-200	ROS0013	5	79	8	220-236	LEI0098	14	37	6	150-170
MCW0063	**2**	**119**	**8**	**132-150**	**ADL0292**	**5**	**83**	**7**	**112-138**	MCW0123	14	45	5	80-90
ADL0217	2	121	4	150-156	MCW0214	5	88	10	268-302	MCW0080	15	49	4	270-280
MCW0065	2	142	6	98-122	MCW0078	5	93	3	135-143	ADL0293	17	26	7	105-119
LEI0089	2	165	6	182-200	LEI0145	5	98	11	303-333	MCW0330	17	41	4	254-286
MCW0039	2	202	5	128-142	MCW0223	5	123	4	177-195	MCW0151	17	57	6	250-266
MCW0034	2	233	7	217-237	**MCW0029**	**5**	**128**	**11**	**137-187**	**ADL0304**	**18**	**7**	**8**	**127-161**
LEI0096	2	233	6	216-240	MCW0081	5	151	2	113-131	MCW0219	18	47	4	224-240
ADL0181	2	241	3	175-179	ADL0166	5	162	9	124-162	MCW0266	19	0	3	163-175
MCW0173	2	243	12	230-272	ADL0298	5	198	6	105-121	MCW0119	20	0	7	102-142
MCW0087	**2**	**252**	**9**	**267-287**	MCW0014	6	50	4	173-187	**ADL0324**	**20**	**18**	**6**	**157-181**
MCW0009	2	261	2	162-172	MCW0250	6	59	5	226-240	ADL0034	20	26	6	111-121
MCW0137	2	273	7	240-264	ADL0230	6	63	6	105-115	SLC2A1	21	71.04	2	293-295
MCW0288	2	275	5	108-118	ADL0159	6	67	10	78-126	ADL0262	23	0	3	105-109
LEI0070	2	379	11	177-213	MCW0120	7	44	10	258-286	MCW0165	23	1	3	114-118
ROS0074	2	302	3	315-321	MCW0201	7	79	4	299-309	ADL0289	23	7	3	173-177
MCW0264	**2**	**320**	**6**	**224-240**	MCW0183	7	86	3	291-319	MCW0301	24	48	6	264-292
GCT0002	2	349	5	154-172	ADL0279	7	92	8	87-115	MCW0285	26	38	7	179-195
MCW0282	2	378	5	286-310	ROS0019	7	101	10	119-147	MCW0069	26	47	7	155-173
LEI0141	2	382	8	220-242	MCW0236	7	109	6	306-328	LEI0074	26	67	6	224-240
MCW0157	2	474	6	285-297	MCW0316	7	127	2	158-186	MCW0300	27	11	3	122-130
MCW0261	**3**	**0**	**8**	**225-251**	ADL0315	7	140	2	245-247	ROS0073	22	0	4	280-292
MCW0083	3	51	5	78-86	MCW0275	8	6	3	128-150	LEI0135	28	0	6	132-142
MCW0222	3	85	4	217-223	ROS0026	8	14	6	109-119	ROS0249	32	20	4	148-162
MCW0212	3	154	3	192-206	MCW0095	8	26	5	72-82	ADL0273	Z	73	4	144-168
ADL0248	3	164	7	122-158	MCW0160	8	35	5	205-229	ADL0201	Z	87	4	138-144
MCW0127	3	167	8	227-247	ADL0154	8	46	8	125-171	MCW0154	Z	95	3	170-186
MCW0103	3	201	2	267-271	ADL0278	8	94	4	111-119	LEI0144	Z	131	4	251-269
MCW0224	3	218	4	292-300	MCW0351	8	105	5	149-159	LEI0121	Z	131	3	257-273
MCW0126	3	231	3	112-132	ROS0078	9	0	16	172-246	LEI0075	Z	165	8	164-200

Table 2. Primer information for 15 selected microsatellite markers

marker	Chr	Dye	Forward (5' - 3')	Reverse (5'-3')
LEI0107	1	NED	GCTGCTCAGAAGCATCTGTGC	ATCATTGCTACACCATGGTTC
MCW0145	1	FAM	ACTTTATTCTCCAAATTTGGCT	AAACACAATGGCAACGGAAAC
MCW0063	2	FAM	GGCTCCAAAAGCTTGTTCTTAGCT	GAAAACCAGTAAAGCTTCTTAC
MCW0087	2	NED	ATTTCTGCAGCCAACTTGGAG	CTCAGGCAGTTCTCAAGAACA
MCW0264	2	FAM	CTTACTTTTCACGACAGAAGC	AGACTGAGTCACACTCGTAAG
MCW0261	3	FAM	GAGCAGTTCATATGAAGTGCAG	GTAGTAGCAGCTACACCAGAG
ADL0292	5	FAM	CCAAATCAGGCAAAACTTCT	AAATGGCCTAAGGATGAGGA
MCW0029	5	VIC	GTGGACACCCATTTGTACCCTATG	CATGCAATTCAGGACCGTGCA
ADL0021	9	PET	GCTCCTCGCTTTGCTCTGAA	GCTTAGCCTCATCTCTTGTA
ADL0259	9	VIC	CTCATTGCAGAGGAAGTTCT	GTAATGGAGGATGCTCAGGT
GCT0016	9	NED	TCCAAGGTTCTCCAGTTC	GGCATAAGGATAGCAACAG
LEI0251	13	NED	GATCTAGAAATGGCTGACTGAC	GGGTTACTCTTATGTTTAATGATGTC
MCW0104	13	FAM	TAGCACAACTCAAGCTGTGAG	AGACTTGCACAGCTGTGTACC
ADL0304	18	FAM	GGGGAGGAACTCTGGAAATG	CCTCATGCTTCGTGCTTTTT
ADL0324	20	NED	TTGCCTCGACGGACCACAAT	GCAGCCCCGCCAAGTAACTG

heterozygosity (He), observed heterozygosity (Ho) and polymorphism information content (PIC) are the vital index for the selection of markers in chicken line discrimination. In this study, we selected 15 microsatellite markers out of the 150 MS markers for the discrimination of Korean native chicken lines. The heterozygosity (He and Ho) and polymorphic information content (PIC) value for the Korean native chicken lines are summarized in Table 3. Among these selected 15 MS markers, LEI0251 is contained highest value of the number of allele, He, Ho and PIC for 12, 0.882, 0.852 and 0.865, respectively. While, MCW0264 marker is showed lowest He and PIC value of 0.709 and 0.648, respectively, but the Ho value lowest in GCT0016 marker. The selection process of MS markers were evaluated for the genetic diversity as of the number of alleles, He, Ho and PIC values range of 6 to 12, 0.709 to 0.882, 0.466 to 0.852 and 0.648 to 0.865, respectively. In order to investigate genetic relationships and breed differentiation, highly polymorphic MS markers were selected. Estimation of genotypic diversity of heterozygosity and PIC value informativeness of MS markers were previously used for the determining the animal breed selection (Berthouly et al., 2008). For the animal traceability, PIC>0.5 and He>0.6 are the most reasonable informative locus for application in genetics (Botstein et al., 1980). In this study, selected 15 MS markers were highly informative among the five chicken lines and these MS markers are appropriate for

Table 3. The statistical analysis of heterozygosity (He and H_O), polymorphism information content (PIC), and *F*-statistics value using selected 15 microsatellite markers among the native chicken lines

Locus	Chr	No. of allele	He	Ho	PIC	*Fst*	*Fit*	*Fis*
LEI0107	1	8	0.77	0.716	0.739	0.1076	0.1001	-0.0083
MCW0145	1	7	0.791	0.727	0.759	0.1141	0.0774	-0.0413
MCW0063	2	8	0.712	0.648	0.665	0.0956	0.1142	0.0206
MCW0087	2	9	0.83	0.761	0.806	0.1219	0.1074	-0.0165
MCW0264	2	6	0.709	0.713	0.648	0.0309	0.0013	-0.0306
MCW0261	3	8	0.842	0.83	0.817	0.1324	0.0455	-0.1002
ADL0292	5	7	0.821	0.716	0.791	0.1345	0.1610	0.0306
MCW0029	5	11	0.789	0.739	0.77	0.1215	0.0761	-0.0517
ADL0021	9	8	0.849	0.795	0.825	0.1111	0.0892	-0.0246
ADL0259	9	9	0.846	0.773	0.826	0.0890	0.0923	0.0036
GCT0016	9	8	0.804	0.466	0.773	0.2473	0.4513	0.2710
LEI0251	13	12	0.882	0.852	0.865	0.0927	0.0538	-0.0429
MCW0104	13	11	0.846	0.659	0.823	0.1965	0.2349	0.0478
ADL0304	18	8	0.768	0.678	0.735	0.1004	0.1449	0.0495
ADL0324	20	6	0.767	0.568	0.723	0.2174	0.2853	0.0868
Total/Mean	8	126/8.4	0.802	0.709	0.771	0.1290	0.1370	0.0093

He = Expected heterozygosity, Ho = Observed heterozygosity, PIC = Polymorphism information content, Fit = Total inbreeding, *Fst* = Genetic distance, *Fis* = Within inbreeding.

Table 4. Pair-wise genetic distance among five chicken lines

	KNC_B	KNC_G	KNC_R	KNC_W
KNC_G	0.156	-	-	-
KNC_R	0.103	0.164	-	-
KNC_W	0.171	0.137	0.137	-
KNC_Y	0.083	0.107	0.111	0.107

discrimination as well.

F-statistics (Wright, 1965) were estimated in a fixation index as genetic differentiation (*Fst*), the global heterozygote deficit among five chicken lines (*Fit*) and the heterozygote deficit within line (*Fis*) among the 15 MS markers (Table 3). Among these markers, estimation of fixation index has been discovered for *Fst*, *Fit* and *Fis* values ranging from 0.0309 to 0.2473, 0.0013 to 0.4513 and -0.1002 to 0.271, respectively. The estimated mean value of the total inbreeding (*Fit*), within line inbreeding (*Fis*) and genetic distance were 0.137, 0.0093 and 0.129, respectively. The high *F*-statistics value was contained in GCT0016 marker of 0.2473, 0.4513 and 0.271 for *Fst*, *Fit* and *Fis*, respectively. While, the lowest value for genetic distance (*Fst*) and total inbreeding (*Fit*) was 0.0309 and 0.0013, respectively, and lowest within inbreeding value of -0.1002 in MCW0261 marker. In addition, pair wise co-ancestry matrix genetic distance was confirmed 0.083 to 0.171 among Korean native chicken lines (Table 4). The highest genetic distance was obtained between KNC_B and KNC_W (17.1%), while the lowest genetic distance was observed between KNC_B and KNC_Y (8.3%).

The expected probability of identity values of 15 MS markers were calculated in random individuals (PI), random half-sib ($PI_{half\text{-}sibs}$) and random sibs (PI_{sibs}), which were estimated as 7.98×10^{-29}, 2.88×10^{-20} and 1.25×10^{-08}, respectively (Table 5). Also, acceptance of marker accuracy for discrimination power was evaluated (Figure 1). The expected probability of chicken lines identity among the genotypes of random individuals (PI), random half-sib ($PI_{half\text{-}sibs}$) and random sibs (PI_{sibs}) were suggested approximately 12 markers. Thus, the expected probability of identity values from 12 MS markers in random individuals (PI), random half-sib ($PI_{half\text{-}sibs}$) and random sibs (PI_{sibs}) were estimated as 1.01×10^{-20}, 3.85×10^{-15} and 1.69×10^{-7}, respectively. Overall, the total expected probability of identity values was 99.9% for the discrimination of Korean native chicken. Our study identified markers in Korean native chicken lines which are applicable to future breeding plans, as well as discrimination markers for these lines.

Table 5. The expected probability values among genotypes of random individuals (PI), random half-sib ($PI_{half\text{-}sibs}$), random sibs (PI_{sibs}) and total expected probability (PE) for discrimination chicken lines using 12 and 15 markers

No. of marker	PI	$PI_{half\text{-}sibs}$	PI_{sibs}	PE
15	7.98×10^{-29}	2.28×10^{-20}	1.25×10^{-8}	0.999858
12	1.01×10^{-20}	3.85×10^{-15}	1.69×10^{-7}	0.999555

Phylogenetic and structure analysis

The unrooted phylogenetic neighbor-joining (NJ) tree was constructed using 15 MS markers that clearly differentiated among five native chicken lines (Figure 2). Based on the equation of Nei et al. (1983), a phylogenetic tree has been estimated by the distribution of allele sharing with genetic distance (*Fst*). In our analysis, the KNC_W line is different from the KNC_B and KNC_R lines. Also, the KNC_G line shows a long genetic distance from KNC_R. However, the KNC_Y line is very close to the KNC_W and KNC_R lines. The dendrogram drawn from the genetic distance matrix using 15 MS markers can also be used for the conservation of Korean native chicken lines. Also, five different lines in Korean native chickens were well discriminated by using these 15 MS markers.

In 1994, the conservation policy for the development of Korean native chicken lines was launched. As a result, five breeds with nine chicken lines were documented. In order to investigate the genetic structure of the five Korean native chicken lines, a structured program of genetic analysis was applied (Figure 3). Based on the line specific clusters, chicken line structure was estimated with a K value of 5. The estimated average individual cluster value in the specific line was accurate to more than 95% (data was not shown). Our structure result for the five Korean native chicken lines indicates around 5% genetic admixture with other lines. In conclusion, our study shows the genetic

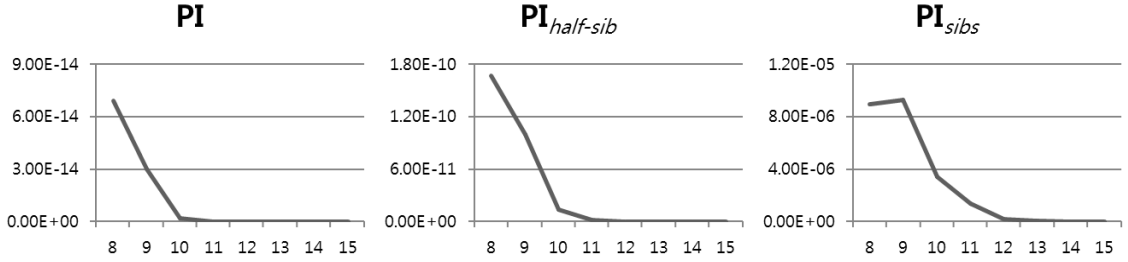

Figure 1. The expected probability of identity values among genotypes of random individuals (PI), random half-sib ($PI_{half\text{-}sibs}$) and random sibs (PI_{sibs}) were suggested markers for discrimination of chicken lines.

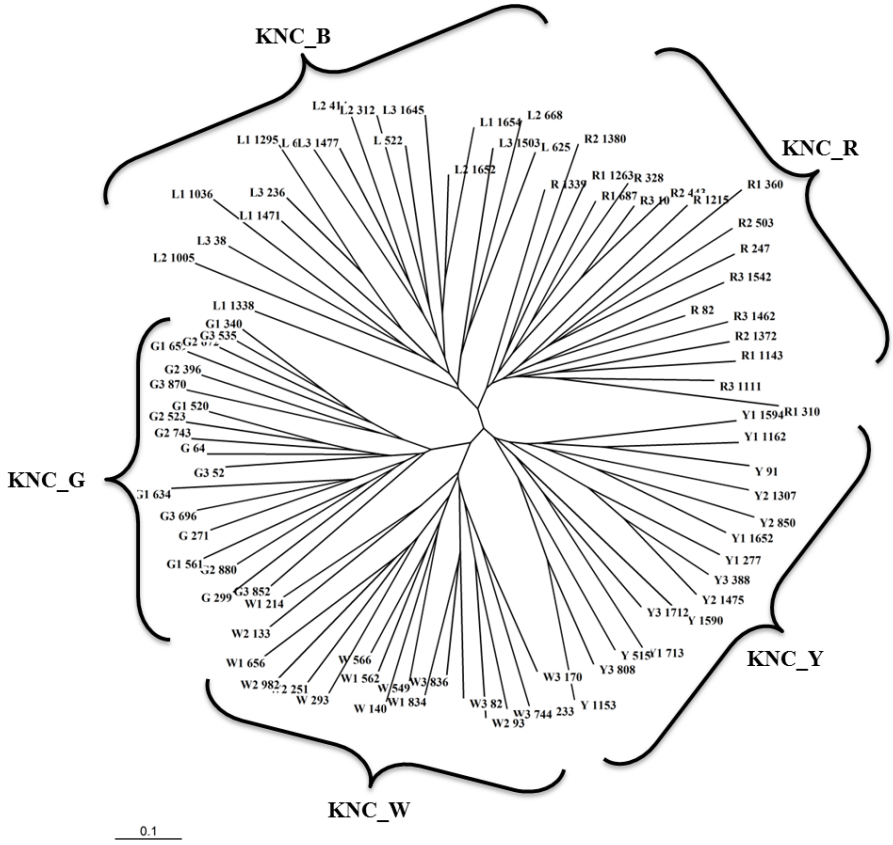

Figure 2. Construction of unrooted phylogenetic neighbor-joining (NJ) tree among the five chicken lines using 15 selected MS markers.

diversity, genetic distance, and population structure among five Korean native chicken lines, using 15 selected MS markers. The maintaining of Korean native chickens with appropriate discrimination markers is the essential for conservation of this breed. Our results indicated that these MS markers will be used to aid the conservation, traceability and future improvement of Korean native chicken lines.

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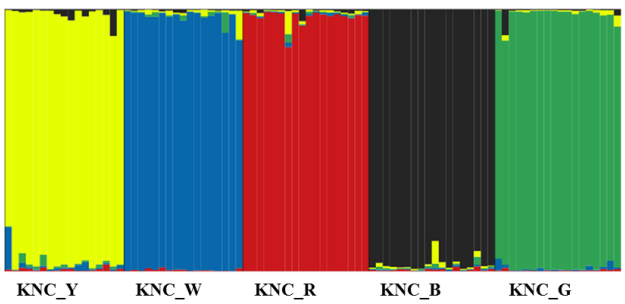

Figure 3. Construction of genetic structure using individual cluster (K value of 5) among the chicken lines.

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