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Author	Eunjin Cho ¹ , Minjun Kim ² , Jae-Hwan Kim ³ , Hee-Jong Roh ³ , Seung Chang Kim ³ , Dae-Hyeok Jin ³ , Dae Cheol Kim ⁴ , and Jun Heon Lee ^{1, 2}
Affiliation	¹ Department of Bio-AI Convergence, Chungnam National University, Daejeon 34134, Korea ² Division of Animal & Dairy Science, Chungnam National University, Daejeon 34134, Korea ³ Animal Genetic Resources Research Center, National Institute of Animal Science, Rural Development Administration, Hamyang 50000, Korea ⁴ Jeju Special Self-Governing Province Livestock Promotion Agency, Jeju 63078, Korea
ORCID (for more information, please visit https://orcid.org)	Eunjin Cho (https://orcid.org/0000-0003-4800-1603) Minjun Kim (https://orcid.org/0000-0002-8173-8431) Jae-Hwan Kim (https://orcid.org/0000-0003-0208-0991) Hee-Jong Roh (https://orcid.org/0000-0003-1458-1937) Seung Chang Kim (https://orcid.org/0000-0001-5724-0599) Dae-Hyeok Jin (https://orcid.org/0000-0001-5091-4271) Dae Cheol Kim (https://orcid.org/0000-0002-4740-0154) Jun Heon Lee (https://orcid.org/0000-0003-3996-9209)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jun Heon Lee
Email address – this is where your proofs will be sent	junheon@cnu.ac.kr
Secondary Email address	
Address	Division of Animal & Dairy Science, Chungnam National University, Daejeon 34134, Korea.
Cell phone number	+82-10-5172-0816
Office phone number	+82-42-821-5779
Fax number	+82-42-825-9754

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Abstract

Genetic diversity analysis is crucial for maintaining and managing genetic resources. Several studies have examined the genetic diversity of Korean domestic chicken (KDC) populations using microsatellite markers, but it is difficult to capture the characteristics of the whole genome in this manner. Hence, this study analyzed the genetic diversity of several KDC populations using high-density single nucleotide polymorphism (SNP) genotype data. We examined 935 birds from 21 KDC populations, including indigenous and adapted Korean native chicken (KNC), Hyunin and Jeju KDC, and Hanhyup commercial KDC populations. A total of 212,420 SNPs of 21 KDC populations were used for calculating genetic distances and fixation index, and for ADMIXTURE analysis. As a result of the analysis, the indigenous KNC groups were genetically closer and more fixed than the other groups. Furthermore, Hyunin and Jeju KDC were similar to the indigenous KNC. In comparison, adapted KNC and Hanhyup KDC populations derived from the same original species were genetically close to each other, but had different genetic structures from the others. In conclusion, this study suggests that continuous evaluation and management are required to prevent a loss of genetic diversity in each group. Basic genetic information is provided that can be used to improve breeds quickly by utilizing the various characteristics of native chickens.

Keywords: Genetic diversity, Population structure, Korean domestic chicken, Single nucleotide polymorphism

Introduction

Genetic diversity depends on the rates of allele loss and fixation, and reflects the balance in emergent genetic variants within populations [1]. It allows animal to survive and adjust to the environmental changes they will face. Genetic diversity is an important aspect of disease prevention and trait enhancement research for a sustainable livestock industry. Commercial breeds with excessive breeding have limited genetic diversity, compared with indigenous breed, since they are frequently bred for conservation without a structured selection procedure [2]. The livestock industry selectively produces commercial animals with high economic benefits, which reduces genetic diversity and could undermine the conservation of indigenous breeds with small populations. Hence, research on genetic diversity is required to maintain and manage their genetic resources.

Various genetic markers have been developed to obtain genetic information. Several studies of genetic diversity have used polymorphic microsatellite (MS) markers throughout the genome [3-5]. Due to their unique properties, however, MS markers do not always accurately reflect the characteristics of the whole genome, and some have high rates of genotyping errors [6]. Furthermore, research using MS markers necessitates much effort and interpretation of the results is highly subjective. The use of single nucleotide polymorphism (SNP) markers could overcome these limitations of MS markers [7]. SNPs are the most common genetic molecular markers throughout the genome and are ideal for large-scale analysis platforms [8]. Various genotyping methods based on SNP assays have recently been developed, and analysis costs are dropping gradually. Therefore, SNP markers are much more effective than MS markers for studying genetic diversity.

Korean domestic chicken (KDC) populations are generally classified into native and commercial breeds. Korean native chicken (KNC) populations are subdivided into five breeds and 12 lines, and the purebred KNC has been preserved by the National Institute of Animal Science (NIAS) in Korea. Six lines of two breeds are indigenous KNCs, including the Gray-brown KNC (NG), Black KNC (NL), Red-brown KNC (NR), White KNC (NW), Yellow-brown KNC (NY), and Yeonsan Ogye (YO). The remaining six lines of the other three breeds are adapted KNCs, which were imported in the 1960s and adapted in Korea for more than seven generations until now and include the Rhode Island Red (NC and ND), Cornish (NH and NS), and Leghorn (NF and NK) lines. In addition to KNCs, which are preserved by NIAS, two local chicken breeds classified as KDCs managed in Korea: Hyunin KDC (HI), and Jeju KDC (J). Although they are preserved in a private institution, their populations are small and they are not managed under an efficient selection system. As well as the native KDCs, Korean poultry breeding companies produce commercial KDCs that have been improved to suit the taste of Koreans. Hanhyup is a representative breeding company that produces several breed lines by improving Rhode Island Red (HS and HW), Cornish (HA, HF, and HH), Plymouth Rock (HG, HV, and HZ), and New Hampshire (HY) lines.

Several studies using MS markers have reported the genetic diversity of indigenous KNCs [9-13]. However, there have been relatively few diversity analyses using large-scale SNP data. Therefore, this study aims to conduct a genetic diversity study using high-density SNP genotype data targeting several KDC populations inhabiting Korea.

Materials and Methods

Samples and genotypes.

Data on three purebred populations were used in this study (Table 1). The first population consisted of 694 KNC birds separated into five breeds and 12 lines, including YO, indigenous and adapted KNC lines. The second population consisted of 47 Korean local chickens from two breeds: Hyunin and Jeju. The third population consisted of 194 Hanhyup commercial KDCs. The first and third populations were genotyped using a 600K chicken SNP array (Affymetrix, Santa Clara, CA, USA) [14], whereas the second population was genotyped using a custom 60K chicken SNP array created by our team.

Data pre-processing and quality control for the genotype data.

A total of 542,717 SNPs and 66,852 SNPs were derived from 600K and 60K arrays, respectively. Genotype data from the 60K SNP array were imputed using Minimac3 and Minimac4 software [15]. After imputation, 468,584 common SNPs were derived from the two SNP arrays. For genotype quality control (QC), PLINK 1.9 software [16] was used with the following cut-offs: genotyping rate $\leq 95\%$, minor allele frequency (MAF) ≤ 0.01 , and Hardy-Weinberg equilibrium (HWE) at $P \leq 0.000001$. Following QC, 212,420 SNPs were subjected to further analysis.

Analysis of genetic diversity.

The genetic distances (GD) among the chicken populations were calculated using Reynolds' equation and the fixation index (F_{ST}) was estimated. The formulas used for these calculations are as follows:

$$\text{Reynolds' GD} = \sqrt{\frac{\sum_l \sum_u (\text{pop1}_u - \text{pop2}_u)^2}{2 \sum_l (1 - \sum_u \text{pop1}_u \text{pop2}_u)}}$$

where u is the total number of alleles, l is the total number of loci, and pop1_u and pop2_u are the respective allele frequencies of populations 1 and 2 [17]. The GD were calculated using the “poppr” R package [18].

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

where H_T is the expected heterozygosity of the total population and H_S is the average heterozygosity of the subpopulation. The F_{ST} values were calculated with the method of Weir and Cockerham [19] using the “SNPRelate” R package [20].

GD and F_{ST} values were visualized as heatmaps using the “pheatmap” R package [21]; the GD values were then used to plot a phylogenetic tree using the “adeigenet” R package [22].

Analysis of population structure.

Principal component analysis (PCA) was performed using PLINK to confirm the genetic clustering of each population with dimensional information on PC1 to PC3, which have the highest explanatory power. The population structure analysis was conducted using ADMIXTURE software, which compares the distribution of the genetic components of each population based on the numbers of random common ancestors with various K values [23]. The two analyses were conducted by dividing the samples into two cases: either all samples in each population were used or ≤ 25 samples were selected randomly from each population. The results of the two analyses were visualized using R software.

Results and Discussion

Population structure from PCA.

PCA was performed on the 600K SNP genotype data for the entire population. Figure 1 shows the genetic clusters for each population. Figure 1a shows the population clusters obtained using all samples. PC1 and PC2 explained 23.65% of the total variance. Indigenous KNC populations, except for the Black KNC (NL), were separated from the other groups, while the adapted KNC populations and Hanhyup commercial KDC populations clustered together. The Hyunin and Jeju KDC populations also tended to cluster individually; however, this was less clear since the sample sizes of each population differed.

Figure 1b shows the PCA result obtained using the ≤ 25 randomly selected samples. Compared with the adapted KNC and Hanhyup populations, the indigenous KNC populations, YO, and two local chicken populations (Hyunin and Jeju) clustered together. Figures 1c and 1d show the clustering result for the KDC population, excluding the adapted KNC and Hanhyup populations derived from imported chicken breeds. Compared with the total population, PC1 and PC2 better explained the genetic distribution of the KDC populations. Figure 1d indicates that the all eight populations could be distinguished on the basis of PC1 and PC3.

Except for indigenous KNC populations, few samples were used for the populations studied. Allelic polymorphism is an important parameter often used to estimate genetic diversity; it is highly reliant on the effective population size [24]. However, obtaining large sample sizes and standardizing unequal sample sizes are often difficult. Therefore, this study was limited to confirming genetic differences between genetically close groups, as shown in Figures 1c and 1d.

Genetic diversity from GD and F_{ST} .

The results of the GD and F_{ST} analyses are shown in Figure 2, and were similar to those of the PCA (Figure 1). The HI and J KDC populations were genetically close to the indigenous KNC group (GD, 0.33 - 0.42; F_{ST} , 0.09 - 0.16). The HG, HV, and HZ groups, which are the same Plymouth Rock chicken breed, were also close to each other, and the HG and HV groups were being especially genetically close.

Although the Hanhyup and NIAS groups included populations originating from the same breed, there was significant GD between them. The NC, ND, and HS, HW populations derived from the Rhode Island Red breed were close genetically, while there was genetic variance between the Hanhyup and NIAS groups. In addition, the HA, HF, HH, and NH, NS populations, which were derived from Cornish breeds, were also distinct from each other. In particular, NH and NS were in the same NIAS group, but were genetically distant. The same result was seen in the phylogenetic tree based on GD (Figure 3). Branches formed according to the origins of each population.

Seo et al. [25] also found genetic differences between the NIAS and Hanhyup populations, which originate from the same species. They found a relatively high F_{is} value in the adapted KNC group compared to the Hanhyup group, which means that the correlation between individuals in the NIAS group was high. These results were attributed to the different breeding selection goals of the two groups. For the NIAS-adapted KNC groups, a limited number of individuals imported into Korea were genetically fixed through indigenization. For the Hanhyup group, on the other hand, genetic fixation resulted from specific mating combinations aiming to produce practical systems.

Population structure from ADMIXTURE.

The ADMIXTURE results for the 21 populations revealed the genetic components and population structures across entire groups. In the two groups using different sample sizes, the optimal cross-validation (CV) error was 0.495 when $K = 8$ using the entire population and 0.508 when 17 using the smaller random subpopulations (Figure 4).

The ADMIXTURE analysis confirmed the results of the phylogenetic tree; $K = 8$ using the total sample (Figure 4a) indicated that all six indigenous KNC populations were distinct. Furthermore, the HI and J KDCs shared common ancestors, comparable to the results of the F_{ST} analysis. Unlike the other groups, it was difficult to classify these two populations as independent groups because of possible hybridization with other breeds, or a lack of individual identification and a breeding plan. The Hanhyup and NIAS groups with

each having the same origin share a common ancestor, based on the results of the phylogenetic tree. Despite the limited number of individuals, the adapted KNC populations (NC, ND, NH, and NS) were clearly divided into groups.

The results at $K = 5$ using the selected samples (Figure 4b) showed that the indigenous KNC populations, except NG and NW, share common ancestors with the HI and J KDCs. Similar results were obtained for other Hanhyup populations in the analysis of all samples. Except for the NG and YO populations, all the chicken populations had a dominant single ancestor when $K = 20$. The ADMIXTURE analysis produced results similar to those of a diversity study using 25 MS markers; using 18 KDC populations, the groups were separated optimally at $K = 15$, and populations from the same ancestral species were classified together [13].

Conclusion

This study performed genetic diversity and population structure analyses using high-density SNP genotype data of various KDC populations. The results of the diversity analysis suggest the existence of genetic diversity among different breeds within the large domestic chicken population in Korea. Furthermore, the results suggest genetic fixation and high population uniformity of the KNC populations and emphasize the need for a systematic selection strategy for the Hyunin and Jeju KDC populations.

In summary, the diversity study conducted on the KDC groups indicates that continuous evaluation and management are required to prevent a decline of genetic diversity in each group. This study provides basic genetic information that can improve breeds quickly by selecting for various characteristics of native chickens.



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Tables and Figures

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Table 1. Summary of the samples used in this study.

Class	Population	Origin	Description	No. Animals
Korean native chicken	NG	Gray-brown KNC	Indigenous KNC	89
	NL	Black KNC		74
	NR	Red-brown KNC		127
	NW	White KNC		94
	NY	Yellow-brown KNC		97
	YO	Yeonsan Ogye		189
	NC	Rhode Island Red	Adapted KNC (imported in 1960s and locally adapted)	6
	ND			6
	NH			6
	NS			6
Korean local chicken	HI	Hyunin KDC	Maintained population in Hyunin Farm	23
	J	Jeju KDC	Maintained population in Jeju	24
Commercial KDC	HA	White Cornish	Maintained population in Hanhyup Farm	20
	HF	Black Cornish		23
	HG	White Plymouth Rock		23
	HH	Brown Cornish		23
	HS	Rhode Island Red		23
	HV	White Plymouth Rock		23
	HW	Rhode Island Red		23
	HY	New Hampshire		21
	HZ	Partridge Plymouth Rock		15
Total				935

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KNC, Korean native chicken; KDC, Korean domestic chicken.

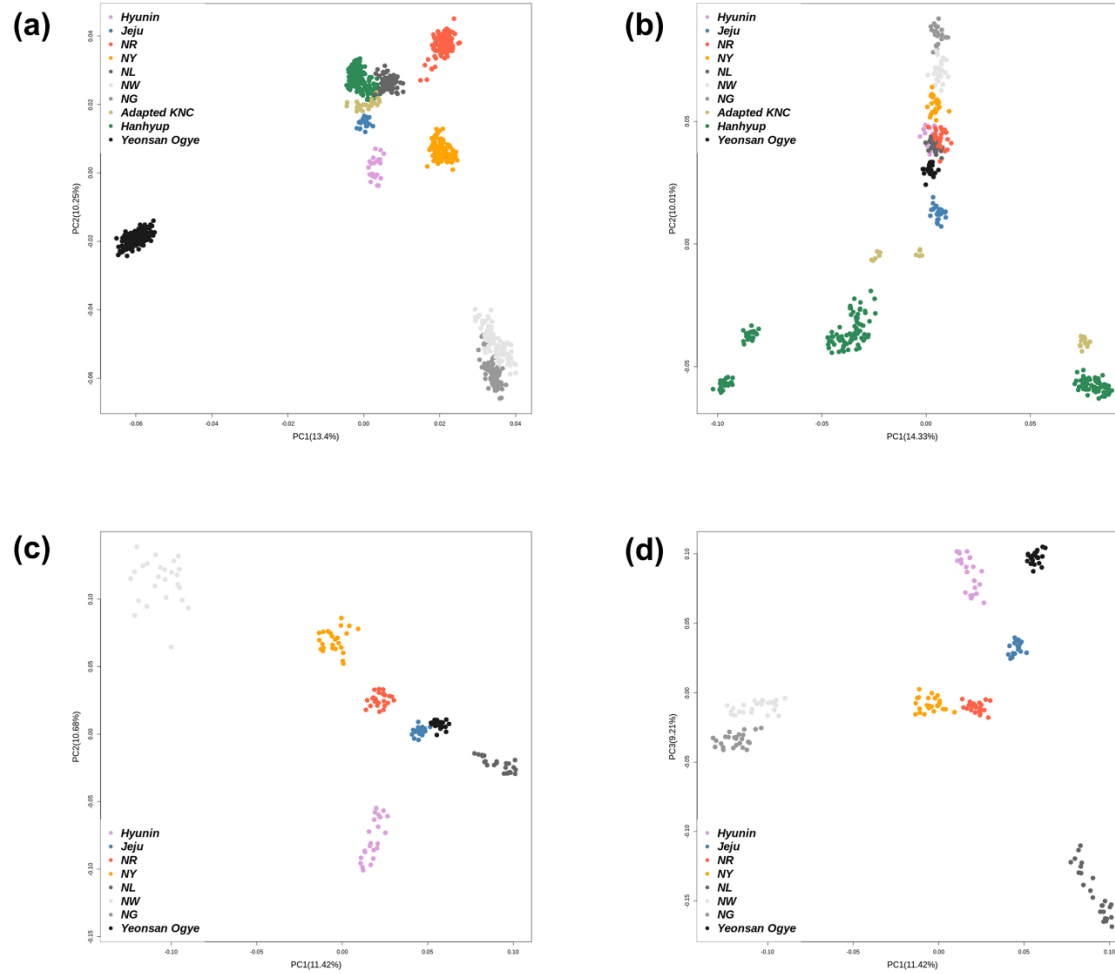


Figure 1. Results of principal component analysis (PCA) using 600K single nucleotide polymorphism genotype data. (a) is the result of PCA using total samples, (b) is the result of PCA using randomly selected samples, and (c) and (d) are the result of PCA without adapted KNC and Hanhyup populations. KNC, Korean native chicken (NR: Red-brown, NY: Yellow-brown, NL: Black, NW: White, NG: Gray-brown).

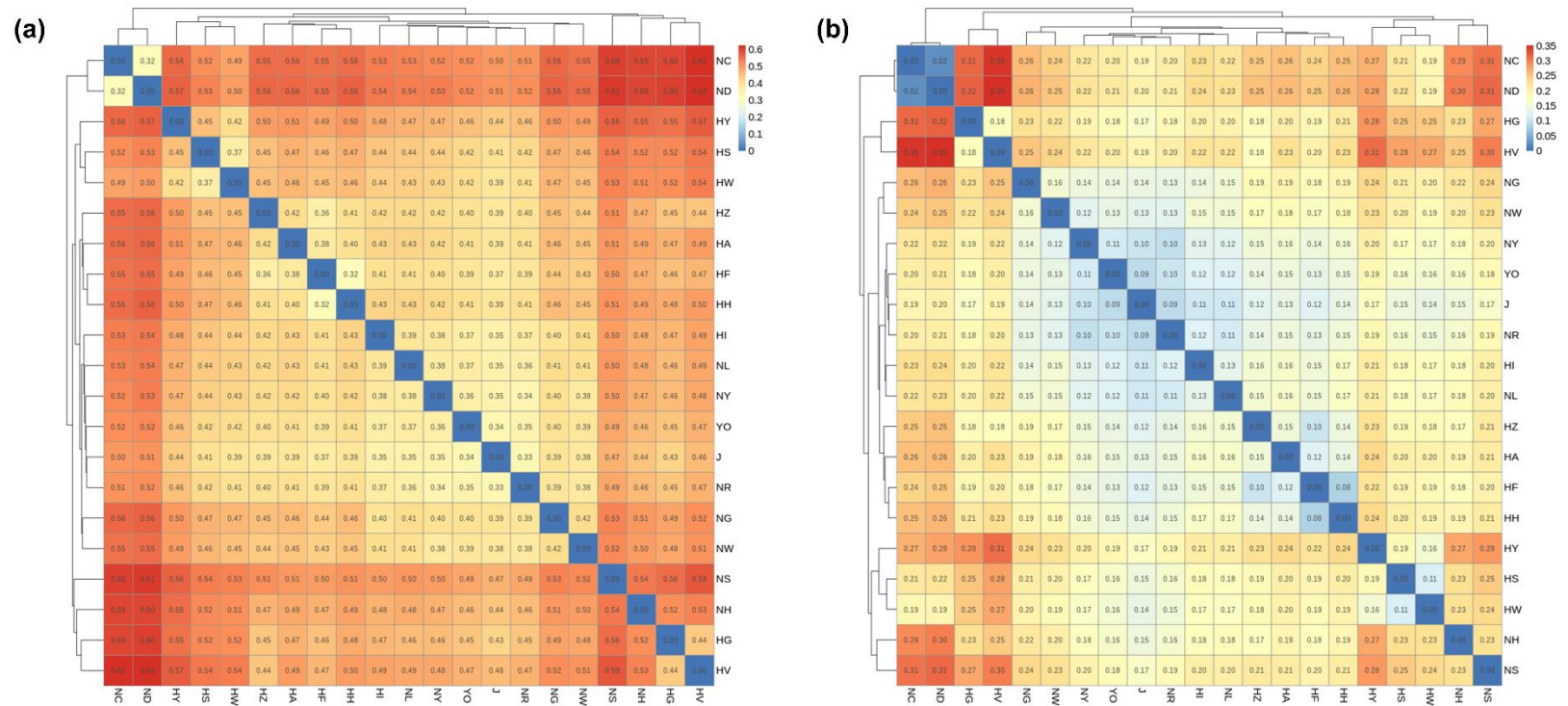


Figure 2. Results of genetic diversity analysis. (a) is the heatmap plot using genetic distance values and (b) is the heatmap plot using fixation index values. KNC, Korean native chicken; KDC, Korean domestic chicken; NG, Gray-brown KNC; NL, Black KNC; NR, Red-brown KNC; NY, Yellow-brown KNC; NC, Rhode Island Red C; ND, Rhode Island Red D; NH, Cornish H; NS, Cornish S; YO, Yeonsan Ogye; HI, Hyunin KDC; J, Jeju KDC; HA, Hanhyup A; HF, Hanhyup F; HG, Hanhyup G; HH, Hanhyup H; HS, Hanhyup S; HV, Hanhyup V; HW, Hanhyup W; HY, Hanhyup Y; HZ, Hanhyup Z.

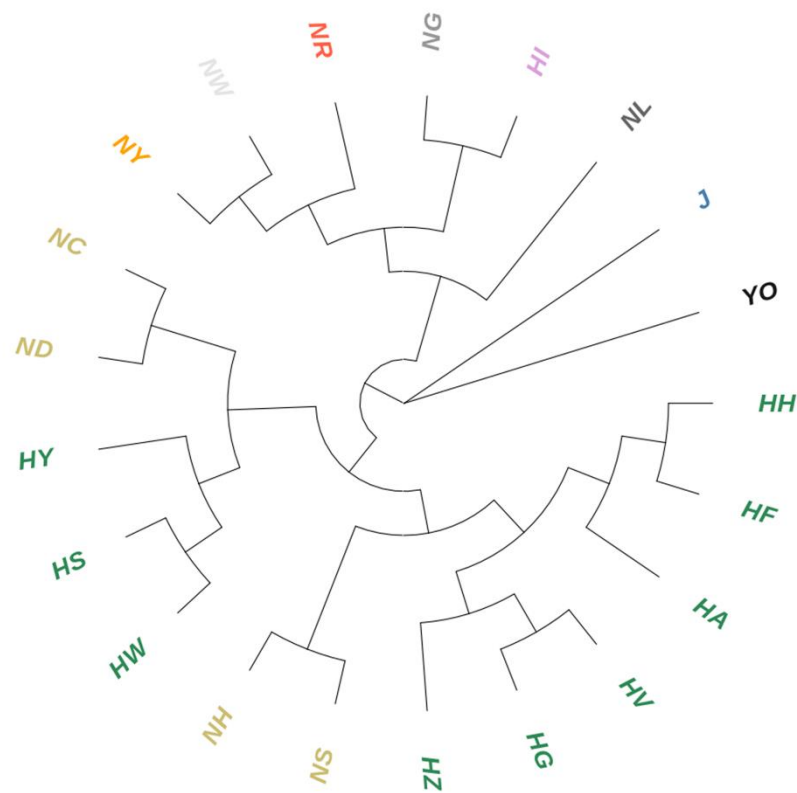


Figure 3. Results of phylogenetic tree using genetic distance values. KNC, Korean native chicken; KDC, Korean domestic chicken; NG, Gray-brown KNC; NL, Black KNC; NR, Red-brown KNC; NY, Yellow-brown KNC; NC, Rhode Island Red C; ND, Rhode Island Red D; NH, Cornish H; NS, Cornish S; YO, Yeonsan Ogye; HI, Hyunin KDC; J, Jeju KDC; HA, Hanhyup A; HF, Hanhyup F; HG, Hanhyup G; HH, Hanhyup H; HS, Hanhyup S; HV, Hanhyup V; HW, Hanhyup W; HY, Hanhyup Y; HZ, Hanhyup Z.

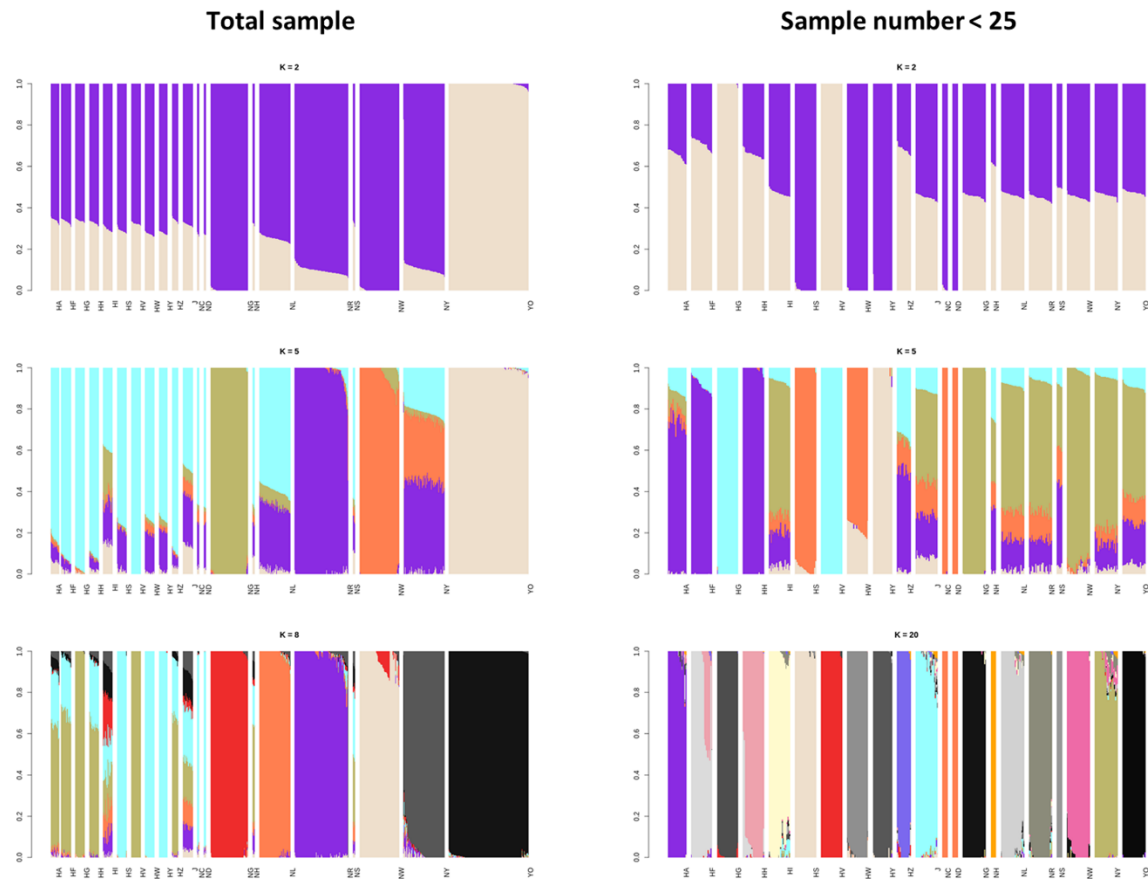


Figure 4. Results of ADMIXTURE analysis. Left plot is the result of ADMIXTURE using total samples, and right plot is the result of ADMIXTURE using randomly selected samples. KNC, Korean native chicken; KDC, Korean domestic chicken; NG, Gray-brown KNC; NL, Black KNC; NR, Red-brown KNC; NY, Yellow-brown KNC; NC, Rhode Island Red C; ND, Rhode Island Red D; NH, Cornish H; NS, Cornish S; YO, Yeonsan Ogye; HI, Hyunin KDC; J, Jeju KDC; HA, Hanhyup A; HF, Hanhyup F; HG, Hanhyup G; HH, Hanhyup H; HS, Hanhyup S; HV, Hanhyup V; HW, Hanhyup W; HY, Hanhyup Y; HZ, Hanhyup Z.