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≪Research Note≫

Population Structure and Genetic Diversity of Seven Chinese Indigenous Chicken Populations in Guizhou Province

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To investigate the population structure and genetic diversity of indigenous chicken breeds in Guizhou, a total of 150 individual samples were collected from 12 breeds, including seven local chicken breeds in Guizhou Province, three Chinese native breeds found in other provinces, and two commercial breeds. The genotype datasets were obtained using a 50K single nucleotide polymorphism array method, and then a series of population analyses were performed. The obtained population parameters and linkage disequilibrium decay indicated a higher degree of genetic diversity in Guizhou chickens than in commercial breeds. Two Guizhou local breeds, Wumeng black-bone and Weining, were clustered with a breed from a neighboring province, Xinwen black-bone, which exhibited similar ancestral composition patterns. A newly found breed, Wumeng crested, had high genetic diversity and displayed genetic differences from other Guizhou breeds. These findings provide insight into the establishment of efficient conservation and utilization programs for Guizhou chicken breeds.

Key words: conservation, genetic diversity, indigenous chickens, population structure

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Introduction

Native breeds are regarded as unique resources for genetic improvement in animal breeding programs. Guizhou Province, located in the temperate and mountainous areas of South China, is rich in domestic chicken populations. According to the latest official survey (China National Commission of Animal Genetic Resources, 2011), several chicken breeds were found to have origins in Guizhou, including Qiandongnan xiaoxiang (QDN), Zhuxiang (ZX), Gaojiao (GJ), Aijiao (AJ), Wumeng black-bone (WB), and Weining (WN). In addition, there are several uninvestigated native breeds in Guizhou Province, including Wumeng-crested (WC), which has the characteristics of both crested head and black bone breeds. These breeds provide valuable genetic resources for scientific research and further genetic improvement in the chicken industry. For example, AJ is a dwarf breed that is caused by autosomal deletion, may be used as potential breeding material for grain-saving layers (Jin *et al*., 2016), while ZX has been a subject of scientific interest for researchers in recent decades because of its excellent cold resistance (Lyimo *et al*., 2014; Wang *et al*., 2015).

However, chickens are probably one of the most under-conserved of all livestock species, as 33% of chicken breeds worldwide are considered endangered (Hoffmann, 2009). Therefore, it is necessary to formulate and implement effective conservation measures for Guizhou local chickens. Effective conservation and utilization of domestic chicken resources rely on the accurate assessment of their genetic diversity and population structure. In recent years, the genetic diversity and population structure of Chinese indigenous chicken populations have been studied using microsatellites (Qu *et al*., 2006; Azimu *et al*., 2018), mtDNA (Zhu *et al*., 2014), and single nucleotide polymorphisms (SNPs) at a genome-wide scale (Chen *et al*., 2018; Mao *et al*., 2019). Until now, the population diversity of Guizhou chickens was unknown. In the present study, we conducted a comprehensive analysis of the genomic architecture of seven Guizhou chicken breeds using a 50K SNP array to investigate their genetic diversity and population structure.

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Materials and Methods

Animal and Genotypic Data

All procedures were approved by the ethics committee of Institute of Poultry Science, Chinese Academy of Agricultural Science (Approval ID: S20181205). Blood samples of indigenous Chinese chickens were obtained from the National Poultry Genetic Resources Biobank (Yangzhou, China) that has collected more than 18,000 blood and tissue samples from native chickens. In total, 109 samples of female individuals were collected, including chickens from seven Guizhou breeds, along with three Chinese indigenous breeds from other provinces: Silkie (SK), Jinhu black-bone (JH), and Xinwen black (XW) (Figs. 1A, S1, and S2; Table S1). Samples of QDN, WB, WN, ZX, and GJ were obtained from our previous study (Tu *et al*., 2004). Samples within each breed were collected from genetically unrelated chickens that did not share a mother or father.

Genomic DNA was isolated from blood samples using the phenol-chloroform method. Genotyping was performed at Compass Biotechnology (Tianjin, China) using the Illumina chicken 50K genotyping array containing 44,561 SNPs. Raw SNP typing data of two commercial breeds, Hubbard (HB) and White Leghorn (WL), from a previously published paper were used in our study (Liu *et al*., 2019).

The acquired SNP datasets were formatted for analysis using PLINK V1.9 (Purcell *et al*., 2007). To increase the accuracy of genotyping, stringent quality control criteria were applied. Individuals with a call rate greater than 95% were retained, and SNP selection was based on the call rate (>95%) and minor allele frequency (>0.01). A final set of 37,837 common SNPs from 150 chickens was used for sub-

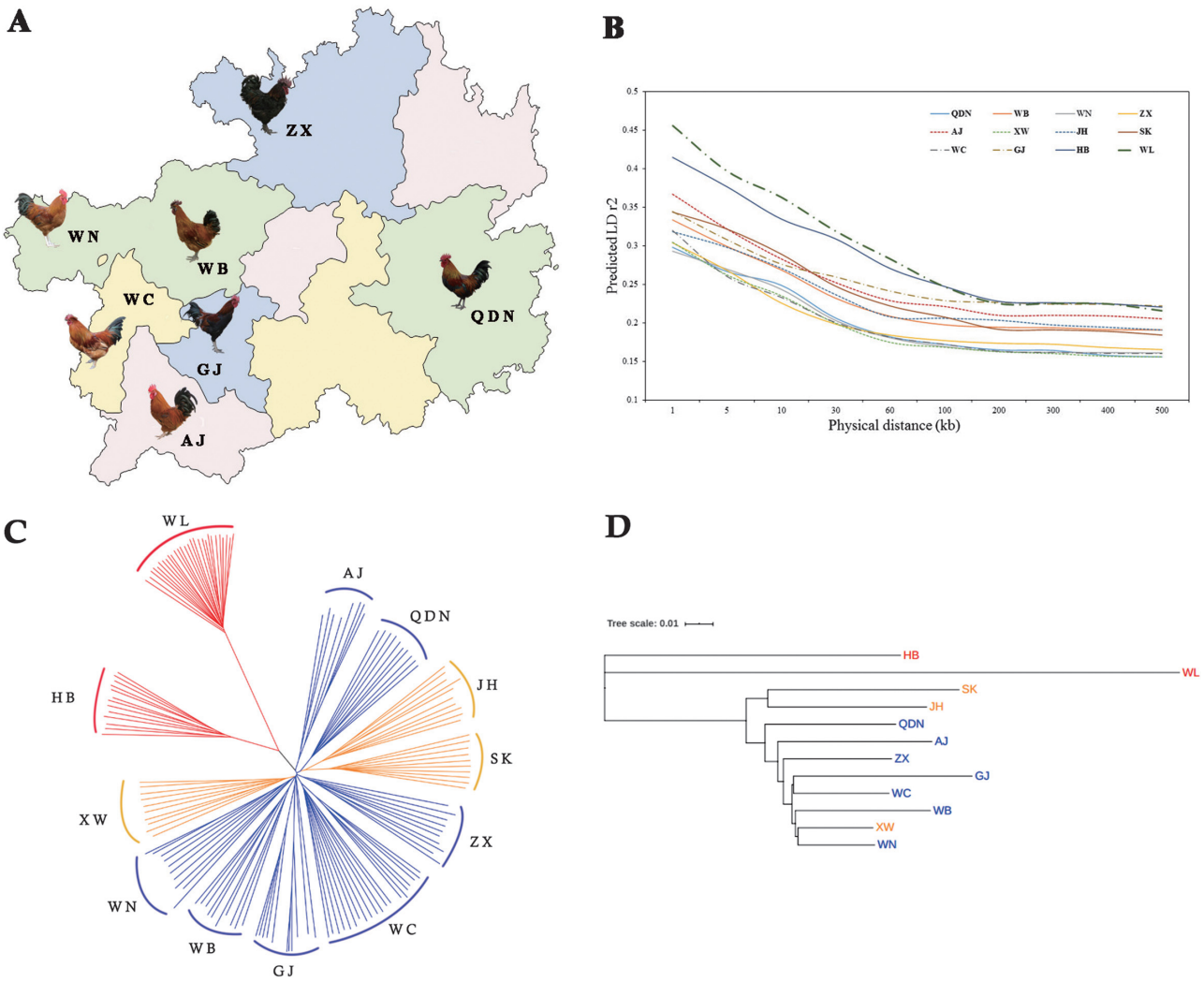

Fig. 1. **Sample location, genetic diversity, and relatedness.** (A) Geographic locations of Guizhou chicken breeds. (B) Average linkage disequilibrium (LD; r2) between markers within an interval of 500 kb in the 12 tested chicken breeds. (C) Neighbor-joining tree based on an identity-by-state matrix. (D) Neighbor-joining tree based on genetic differentiation (F_{ST}) values among breeds.

sequent analysis. The variation data reported in this paper have been deposited in the Genome Variation Map (GVM, http://bigd.big.ac.cn/gvm/home) under accession number GVM000045.

Heterozygosity and Linkage Disequilibrium

To compare the genetic diversity of different chicken populations, we calculated the expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) separately for these breeds using PLINK V1.9. Link-

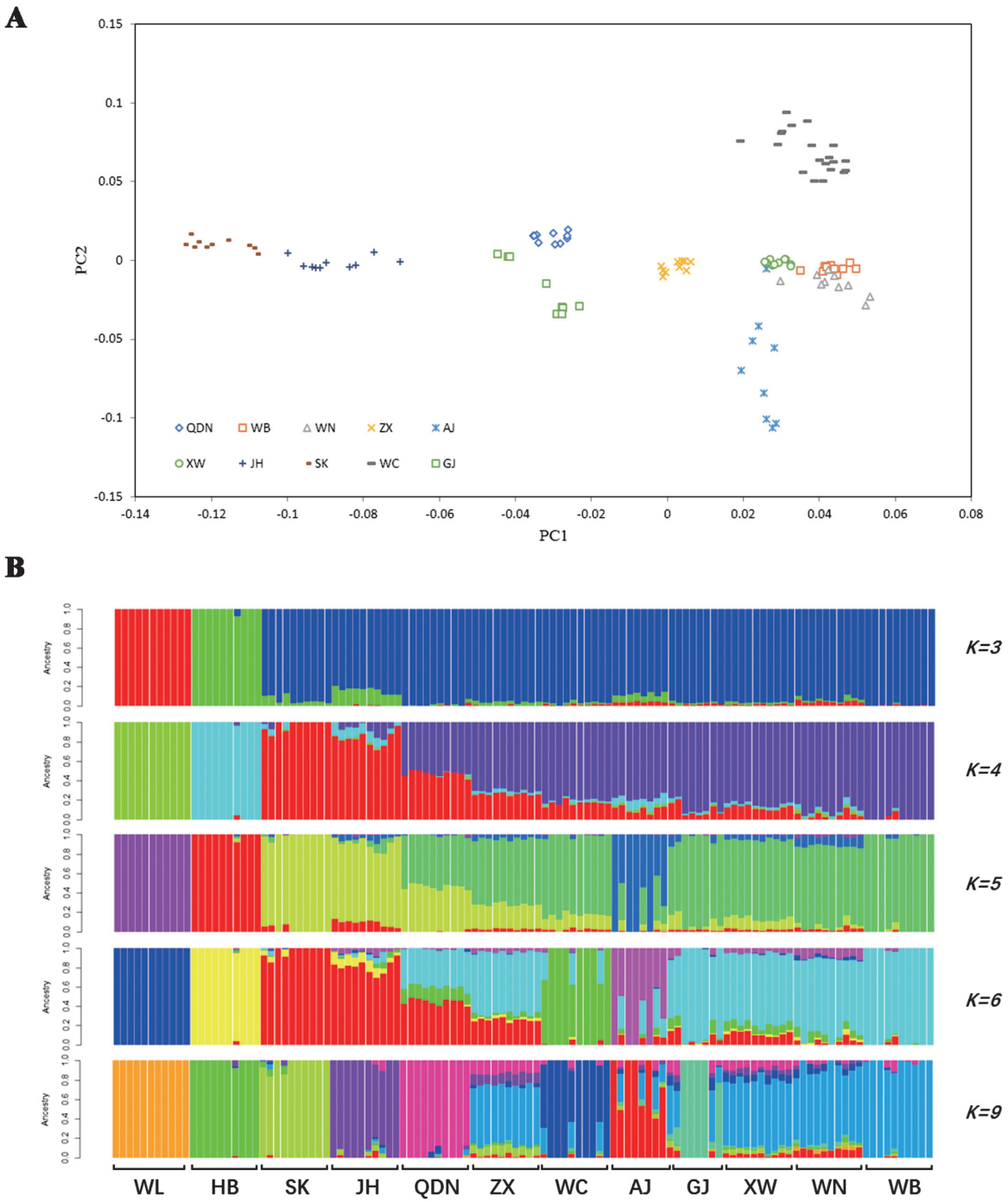

Fig. 2. **Population structure analyses of all chickens.** (A) Principal component plot of seven Guizhou chicken breeds. (B) Population structure plots for all chicken populations with K values of 3, 4, 5, 6, and 9. The same color indicates groups with the same ancestry.

age disequilibrium (LD) between markers was calculated with a 500-kb distance using PLINK V1.9.

Construction of Neighbor-joining Tree

To infer the population structure of the seven Guizhou chicken breeds, we used PLINK V1.9 to construct an identity-by-state (IBS) matrix that quantified the genetic similarity between individuals and to compute differentiation (F_{ST}) between each pair of breeds (Weir and Cockerham, 1984). The IBS and F_{ST} matrices were then utilized to construct neighbor-joining (NJ) trees of individuals and breeds, respectively, using MEGA7 software (Kumar *et al*., 2016). The NJ trees were displayed using the online software ITOL V4 (Letunic and Bork, 2019).

Principal Component Analysis (PCA) and Admixture Analysis

PCA was carried out using GCTA4 (Yang *et al*., 2011) to illustrate population relationships. To further understand the degree of admixture in the populations, we used ADMIXTURE V1.23 (Alexander *et al*., 2009) to investigate the genetic structure of all individuals.

Results and Discussion

The genetic diversity parameters obtained for the 12 different chicken breeds are summarized in Table S1. The inbreeding coefficient was positive in all breeds and varied in the range of 0.085 (ZX) to 0.223 (HB). The expected heterozygosity varied from 0.256 (WL) to 0.346 (ZX), whereas the observed heterozygosity ranged from 0.305 (HB) to 0.338 (WN, WC) across all populations. The Chinese indigenous chickens had higher H_E and H_O and lower F_{IS} values than commercial populations.

The pattern of LD decay can provide information about the evolution of a population. Therefore, comparing the extent of LD between populations can elucidate the overall diversity level. In this study, all Chinese indigenous chickens had a lower LD level than those in the commercial populations (Fig. 1B). In addition, native chicken breeds were divided into two groups according to the LD pattern: lower for XW, WN, ZX, QDN, and WC; and higher for AJ, SK, GJ, WB, and JH. These results suggest that indigenous Chinese chickens, including Guizhou breeds, have a higher genetic diversity than their commercial counterparts, which is consistent with the results of previous studies (Qu *et al*., 2006; Chen *et al*., 2018). However, WB and SK displayed relatively low nucleotide variability, which might be explained by the fact that these two breeds have experienced intensive selection.

In the NJ tree based on genome-wide allele sharing, all individuals of the same breed clustered together (Fig. 1C). The NJ trees showed that the Chinese native breeds and the commercial populations were classified into two separate clades (Figs. 1C and 1D). Among the Guizhou breeds, QDN and AJ were clustered into distinct branches that were distant from other populations; whereas WN, WB, and XW clustered together to form a major branch in the NJ trees. PCA was also conducted to identify the relationship between populations and individuals. In the PCA plot, PC1 clearly separated Chinese native breeds from the commercial breeds (Fig. S3). Within Guizhou breeds, PC1 and PC2 were identified in QDN, GJ, AJ, WC, and ZX, whereas WB, WN, and XW did not form separate clusters, which is compatible with the NJ clustering results (Fig. 2A).

The differentiation of the populations was also examined on the basis of Bayesian clustering analysis using the ADMIXTURE program. The clustering results for the 12 tested breeds with K values from 2 to 12 are shown in Figs. 2B and S4, and the lowest cross-validation error was found when K was 4 (Fig. S5). When K was 3, the Chinese native breeds and commercial breeds appeared as two differentiated clusters. When K was 4, SK and JH were separated from the other native breeds. When the K value ranged from 5 to 9, AJ chickens formed the first independent population among Guizhou chickens, followed by WC, GJ, and QDN. When K was 9, a similar ancestral structure was displayed for WN, XW, and WB.

We found that XW, WB, and WN had a closer phylogenetic relationship with each other than with any other breeds. They were clustered together in both the NJ trees and PCA plot and exhibited similar ancestral composition patterns. Considering their geographic proximity to each other, these results revealed that the three breeds were likely derived from a common ancestral population. In contrast, QDN, GJ, WC, and AJ exhibited a clear divergence from the other Guizhou breeds and represented old ancestral lineages of Guizhou indigenous chickens. Although WC is not an official chicken breed at present, this study could provide reliable evidence for the certification of this breed. Based on these results, governments should implement conservation strategies to maintain the diversity and characteristics of Guizhou chickens. Additionally, our results demonstrated that Guizhou chickens might have undergone little selective pressure, indicating that the potential of these genetic resources needs to be explored further.

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Conflicts of Interest

The authors declared that they have no conflicts of interest to this work.

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