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# Genetic Diversity and Population Structure of Five Chinese Local Cattle Breeds in Guizhou Province, China

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## ABSTRACT

**Background:** This project analyzed the genetic diversity and population structure of five local cattle breeds in Guizhou, in order to implement the conservation plan for local cattle breeds and promote their sustainability.

**Methods:** The experiments used a 100K SNP chip to obtain genotype data from the local cattle breeds, which was compared with that of two introduced breeds (Red Angus and Limousin) as controls (N=112). The genetic diversity, LD decay, PCA and phylogenetic tree were analyzed.

**Result:** The effective number of alleles (Ne), nucleotide diversity (Pi) and LD decay rate of the local cattle breeds were higher than those of introduced breeds. The results of PCA, phylogenetic tree, G matrixand genetic distance analyses indicated that the five local breeds were on the same evolutionary branches without obvious clustering. The proportion of polymorphic markers (Pn), genetic diversityand genetic differences in the Weining cattle population was much higher than that of other populations. These findings provide a reference for the genetic structure and efficient protection and utilization of local cattle in Guizhou.

**Key words:** Genetic diversity, Genotyping array, Local cattle breeds, Population structure.

## INTRODUCTION

Guizhou, located in southwest China, is characterized by highland mountains with rich and lush vegetation. There are five local cattle breeds in this region: Sinan (SN), Guanling (GL), Liping (LP), Wuchuan black(WCH) and Weining (WN) (Records of livestock and poultry breeds in Guizhou, 1993); Journal of China's Livestock and Poultry Genetic Resources-Cattle Resources, 2011). These breeds, characterized by tender meat, finer body and thinner skin, serve as valuable resource breeds in the cattle breed resource gene bank. However, the economic benefits of these local cattle breeds are far inferior to those of exotic or hybrid breeds. Years of crossbreeding have led to a population decline, with only small quantities now farmed in rural areas. For example, the genetic resources of Wuchuan Black cattle are at risk of extinction (Liang *et al*., 2018). More importantly, this trend is likely to continue and further affect local breeds with the intensification of crossbreeding.

In recent years, population genetic background analysis has become an important tool to evaluate and study the genetic structure of animal populations. With the development of SNP chip and sequencing technology, researchers can now understand the genetic diversity and genetic links between different populations more comprehensively. For the first time, the complete mitochondrial genome sequence of 15 pooled samples belonging to five cattle breeds of Tamil Nadu was carried out using the Illumina platform (Vani *et al*., 2022). Strucken *et al.* (2021) used a 777K chip to study the genetic diversity and population structure of 15 local Indian cattle breeds and found that Indian Zebu cattle had higher genetic diversity than local Indian common cattle (Strucken *et al*., 2021).

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Similarly, the Illumina BovineHD BeadChip genotyping array was used to describe the genetic variability and divergence among 7 important autochthonous Spanish beef cattle breeds (Cañas-Álvarez *et al*., 2015). Garcia *et al.* (2023) established an SNPs panel for pedigree reconstruction using microarrays of different densities and evaluated the genomic relationship, coefficient of the inferred pedigree and population structure in Gir cattle. Chen *et al.* (2023) constructed the Yunling cattle standard reference genome and aligned the whole genomes of 129 Yunling cattle individuals to the constructed reference genome to estimate the current genetic status of Yunling cattle in Yunnan Province, China. The mitochondrial DNA control region of the great Indian rhinoceros was studied using non-invasive dung samples collected from wild populations in three protected areas in Assam. The study showed a high level of genetic diversity among the rhinoceros populations

in these three habitats (Strucken *et al*, 2021). A total of 96 individuals from 5 breeds were genotyped using six microsatellite markers of cattle native to Chongqing and 4 introduced breeds (Ni *et al*., 2018). The Guizhou cattle breeds originated from *Bos taurus* and *Bos indicus* in nearly equal measure (Liu *et al*., 2006). Studies have showed that the Weining and Wuchuan black cattle have high heterozygosity and smaller genetic distances. 71.3% of the genetic differentiation was found among Guizhou local cattle breeds, while 28.7% of the genetic variation was found within breeds (Wang *et al*., 2008; Yang *et al*., 2016). The ANGPTL4 and POMC genes have a rich genetic diversity in Guanling cattle where growth traits such as body length showed significant differences between different alleles (Xu *et al*., 2020). However, the genetic structure among the five local cattle breeds in Guizhou has not been studied. This study employed the Illumina Bovine 100 K gene chip for rapid population screening to conduct a comprehensive analysis of the population structure of the five local cattle breeds in Guizhou. Utilizing and maintaining the production advantages of these breeds play an important role in cattle breeding. The study provides a theoretical basis for the protection and utilization of local cattle breeds in Guizhou.

## MATERIALS AND METHODS

### Materials collection

All animal experiments in the study were reviewed and approved by the Subcommittee of the Experimental Animal Ethics of Guizhou University. From July to September 2022, the test samples were randomly selected from the core breeding areas in Guizhou Province and conformed to the breed standards. Blood collection cards were used to collect the blood of the test cattle and were placed in sampling storage boxes. They were then transported back to the laboratory and stored at -20°C for further use. A total of 112 individual samples were collected, including 20 samples of each local breed (Sinan (SN), Guanling (GL), Liping (LP), Wuchuan black (WCH) and Weining (WN), In addition, 6 samples of each of the introduced breeds (Red Angus and Limousin) were collected from the Guizhou Provincial Bull Breeding Station.

### Genotype date selection

Genomic DNA was extracted by using TIANamp genomic DNA kit following the manufacturer's protocol (TIANGEN Biotech, Beijing). Genotyping of 63,309 SNPs was performed using the Illumina Bovine 100K Genotyping Array at Beijing Compass Agritechnology Co., Ltd. (Beijing, China) Quality control on the genotype data was performed using the Plink V1.9 (Purcell *et al*., 2007) software and only loci which with the best typing quality were retained. Genotype data quality control standards and SNP selection were performed based on the SNP detection rate (call rate) of >90% and the minimum allele frequency (MAF) of >0.01, The Hardy-Weinberg (P) value>0.000001 was also used as control standards for genotype data selection. Finally, 61,104 SNPs from 112 cattle were identified for subsequent analysis.

### Genetic diversity and population structure

PLINK V1.9 was used to calculate the proportion of polymorphic markers (Pn), expected heterozygosity (He), observed heterozygosity (Ho), nucleotide diversity (Pi), effective number of alleles (Ne) and population differentiation index ($F_{ST}$) of the populations for analysis of the genetic diversity of the seven test breeds (Barbato *et al*., 2015; Sved 1971; Hao *et al*., 2017; Li *et al*., 2014). PLINK V1.9 was also used to calculate LD with a marker distance of 200 kb to analyze the intensity of selection and the level of genetic material polymorphism of the different breed groups. Principal component analysis (Admixture Analysis, PCA) was performed using the GCTA software (Yang *et al*., 2011), to illuminate the relationships between the seven breeds. To further understand the degree of mixing of the population, Admixture V1.23 (Alexander *et al*., 2009) was used to screen the genetic structure of all individuals. Here, G matrix (V2) was used to construct a genomic relationship matrix using genome-wide markers (VanRaden, 2008). The genetic similarity between individuals was quantified using PLINK V1.9, to construct an identity-by-state (IBS) matrix and to calculate the differentiation ($F_{ST}$) between each pair of breeds (Weir, 1984). The kinship relationships between individuals in the seven study groups were also determined through IBS distance matrix analysis. These experiments were based on MEGA7 software (Kumar, 2016) which used SNP genotyping data to construct a phylogenetic tree using the neighbor-joining method (Neighbor-Joining, NJ) and online software ITOLV4 was used to display the tree (Letunic, 2019).

## RESULTS AND DISCUSSION

Population genetic diversity is usually presented through the observed heterozygosity (Ho) and expected heterozygosity (He) values of the population, Lower Ho values indicate a smaller genetic diversity, while higher values indicate genetic consistency (Li *et al*., 2020). In our study, the effective number of alleles (Ne), proportion of polymorphic markers (Pn) and nucleotide diversity (Pi) of the local breeds were found to be greater. This is likely because the local cattle breeds have not undergone thorough systematic breeding. The results are consistent with previous studies about Guizhou local cattle breeds (Zhang, 2006; Yang *et al*., 2016; Liu *et al*., 2005; Xu, 2024). On the contrary, the introduced breeds have lower genetic diversity. The samples of the introduced breeds were collected from the Guizhou Provincial Breeding Bull Station, where they are all pedigree bulls with high purity (Table 1 and Fig 1). The pattern of LD decay can provide information about population evolutionand the degree of LD between populations can illustrate the overall level of diversity (Przeworski, 2002). Generally, a slower LD decay rate

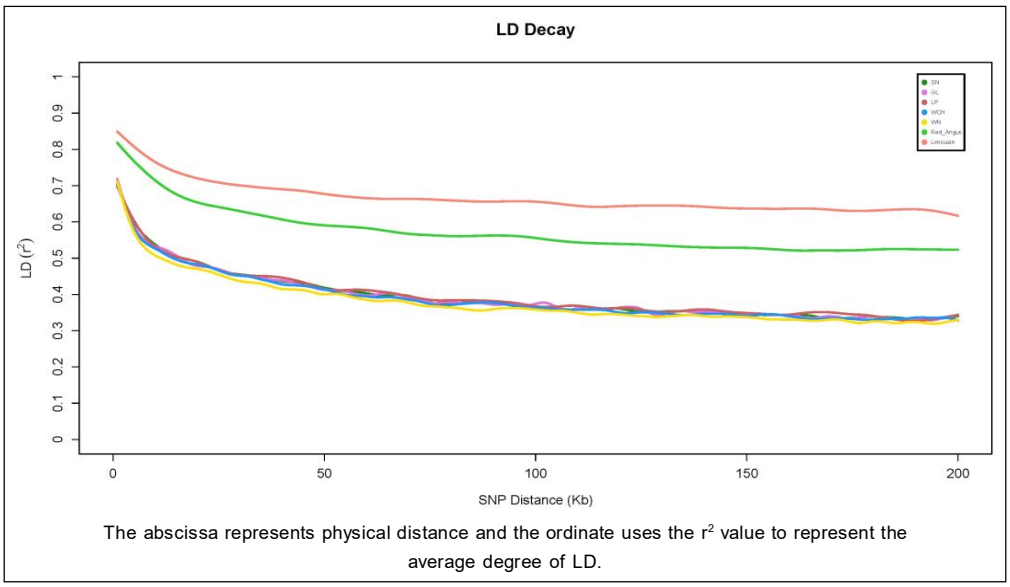
indicates that the population was subject to selection and the stronger the degree of selection. The difference in the degree of selection between each subpopulation was found by comparing their LD decay rates (Rafalski, 2004). The LD levels of local cattle SN, GL, LP, WCH and WN had a lower LD level. These results indicated that Guizhou local cattle breeds have a higher genetic diversity than introduced breeds, which is expected and consistent with previous work (Yang *et al*., 2016).

The G matrix, IBS distance matrix and $F_{ST}$ value analyses indicated an absence of close genetic relationships between the introduced breeds and the five local cattle populations. The statistics of group differentiation indices by cattle breeds indicated a small degree of differentiation (between 0.179 and 0.342) (Table 2). The $F_{ST}$ values indicated that the WN population possessed the highest population differentiation index. The grouping of all individuals by breed indicated that the Guizhou local cattle and introduced breeds were clearly grouped into two independent branches. In particular, the WCH, LP and WN populations were on separate branches that were distant from the GL and SN populations (Fig 2). The genetic distances and genetic relationships between the introduced breeds and native breeds were farther apart, while the WN group was also genetically more separated from the SN, LP, GL and WCH breeds. This demonstrated that the local breeds were less affected by introduced breeds. The distant genetic relationship between the WN cattle and other local breeds is probably due to that Weining cattle were in the geographically distant alpine mountainous area (Guizhou Plateau altitude of 1000-2800 m.) (He *et al*., 2013).

In the evolutionary tree, all individuals of the same breeds clustered together based on allele sharing. The local cattle breeds and introduced breeds were divided into two independent branches. Some WCH and WN individuals were present in branches distant from GL, LP and SN, while the three latter breeds were clustered together to form a major branch in the NJ tree. Phenotypically, the WCH population has a black coat and is geographically more isolated compared to the other local breeds, which may explain its distinct clustering. In the PCA diagram, PC1 clearly distinguished the Guizhou local breeds from the introduced breeds, suggesting that the different cattle breed populations vary widely in their genetic backgrounds. The SN, GL, LP and WCH individuals were randomly gathered together without obvious clustering.

**Table 1:** Genetic diversity statistics for each population.

| Group ID | Ne | Pn | He | Ho | Pi |
|---|---|---|---|---|---|
| SN | 4.30 | 0.881 | 0.321 | 0.320 | 9.967E-06 |
| GL | 4.30 | 0.895 | 0.322 | 0.314 | 1.001E-05 |
| LP | 4.30 | 0.877 | 0.309 | 0.306 | 9.591E-06 |
| WCH | 4.00 | 0.899 | 0.322 | 0.322 | 1.013E-05 |
| WN | 3.90 | 0.940 | 0.358 | 0.365 | 1.125E-05 |
| Red Angus | 1.00 | 0.902 | 0.390 | 0.394 | 8.423E-06 |
| Limousin | 1.00 | 0.890 | 0.381 | 0.391 | 5.356E-06 |




The abscissa represents physical distance and the ordinate uses the $r^2$ value to represent the average degree of LD.

**Fig 1:** Linkage disequilibrium (LD) attenuation.

**Table 2:** Statistics of group differentiation indices by cattle breed.

| | SN | GL | LP | WCH | WN | Red Angus |
|---|---|---|---|---|---|---|
| GL | 0.024 | | | | | |
| LP | 0.025 | 0.025 | | | | |
| WCH | 0.023 | 0.024 | 0.024 | | | |
| WN | 0.033 | 0.031 | 0.038 | 0.031 | | |
| Red Angus | 0.324 | 0.321 | 0.339 | 0.320 | 0.268 | |
| Limousin | 0.342 | 0.341 | 0.358 | 0.338 | 0.283 | 0.179 |

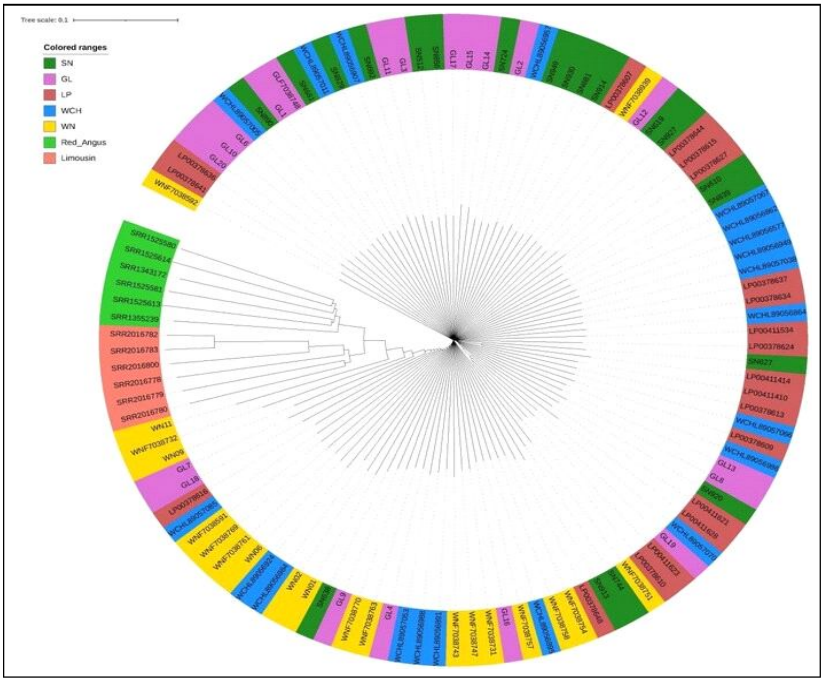



**Fig 2:** Evolutionary tree analysis.

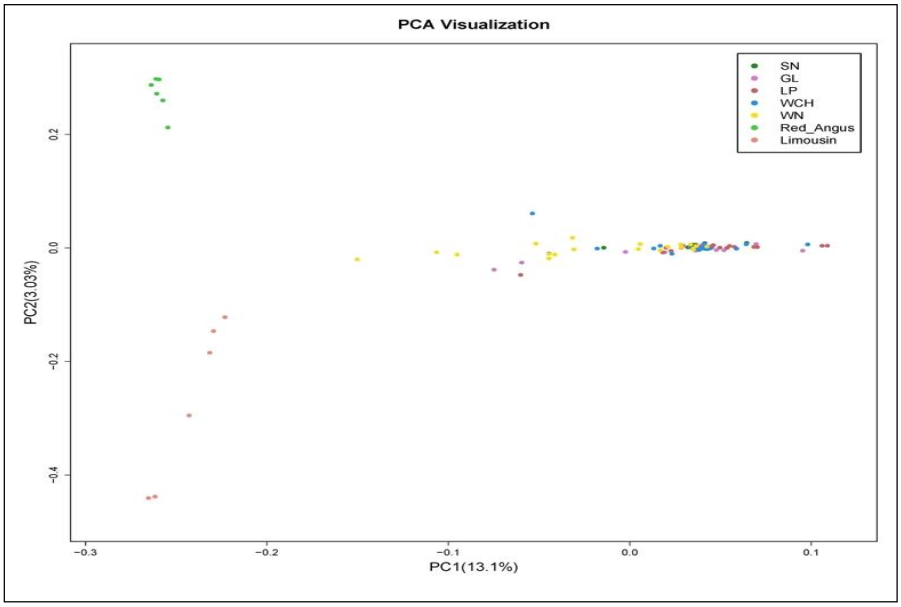



**Fig 3:** PCA analysis of test cattle used for this study.

In contrast, the WN individuals were obviously dispersed from SN, GL, LP and WCH groups, indicating that the genetic background of the SN, GL, LP and WCH groups is similar or that there has been mutual hybridization. However, the genetic background of WN remained relatively distant. These findings are consistent with the results of studies on Guizhou local cattle breeds (He *et al*., 1999). The NJ tree and PCA plots showed closer genetic relationships between the GL, LP and SN breeds, which is expected given their geographical proximity. These results suggest that these three breeds most likely originated from a common ancestral population or have interbred with each other due to geographical proximity. The Red Angus and Limousin breeds were grouped separately, which was consistent with the NJ clustering results. which is consistent with the phylogenetic and PCA results (Fig 2 and Fig 3). The clustering of the breeds was apparent when K values ranged from 2 to 8 in the seven populations. When K = 2, local breeds and introduced breeds were present in two differentiated clusters. When K = 4, Red Angus and Limousin were divided into two subgroups and were separated from the local breeds. When K ranged from 2 to 8, SN, GL, LP, WCH and WN did not separate into groups indicating that genetic exchange may have occurred between the ancestors of these five groups, which is in accordance with our previous study (Xu, 2024). These results were consistent with the PCA analysis (Fig 3).

## CONCLUSION

The results of the current study indicated that the SN, GL, LP, WCH and WN cattle breeds were closely related. The ancestors of these five groups may have had genetic exchanges or have interbred due to geographical proximity. In contrast, the genetic relationships between the introduced and the local breeds in Guizhou were relatively distant, indicating that the local cattle in Guizhou were less affected by the introduced breeds. Guizhou local cattle may have experienced unique selection pressuresand the potential of these genetic resources needs to be conserved and further developed. Meanwhile, the SNP chip can serve as a valuable tool for selecting purebred cattle.

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### Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

### Informed consent

All animal procedures for experiments were approved by the Subcommittee of the Experimetal Animal Ethics of Guizhou University and handling techniques were approved by the University of Animal Care Committee.

### Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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