

Genetic analysis of Korean horse and Kyrgyz native horses using a Equine SNP chip

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말 SNP 칩을 이용한 한국말과 키르기스스탄 재래마의 유전적 분석

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Abstracts

The Kyrgyzstan's native horses are an important part of Kyrgyzstan's cultural heritage and biodiversity. Understanding the genetic complexity of these horses can aid conservation efforts by identifying unique genetic traits and developing targeted conservation strategies. Molecular biological characterization can play an important role in the conservation of Kyrgyzstan's indigenous population by ensuring genetic diversity. Genetic differences between each breed were studied through molecular biological analysis of Jeju horses (JEJU), Jeju riding horses (RIDE), Thoroughbreds (THOR) and Kyrgyz native horses (KYRG) using the Illumina GGP Equine Infinium HD Chip. Principal component analysis confirmed that KYRG, JEJU, and THOR clustered each breed independently, and only RIDE was spread in the areas of JEJU and THOR. KYRG has been shown to maintain an independent cluster of populations due to geographical isolation, and in particular, JEJU and RIDE have been shown to be highly genetically related. By calculating the allele frequency, the genetic distance information for each individual was constructed as a Hitmap. The genetic distance of the four breeds confirmed that the THOR breed was relatively far genetic compared to other breeds, and JEJU confirmed that some RIDE and KYRG breeds had a relatively closer genetic distance than the THOR breed. As a result of individual genetic distance, RIDE confirmed that the genetic distance between individuals was relatively close, and KYRG confirmed that the genetic distance between individuals was farther than other breeds. It is considered that the KYRG breed has a lower degree of genetic fixation than other breeds and that genetic diversity is well maintained.

1. Introduction

Kyrgyzstan native horse (KYRG) has evolved to be suitable for Kyrgyzstan with various unique characteristics while adapting to the harsh environment of the region. KYRG is one of the most important animals from a historical, cultural and economic point of view among the various indigenous species. This study was to evaluate the genetic characteristics of Korean Horse and KYRG using a Equine SNP chip.

2. Materials and Methods

2.1 Animals and genotype assays

The animals used in this study were Jeju horses (JEJU), Jeju riding horses (RIDE), thoroughbreds (THOR), and Kyrgyzstan breeds. The blood of JEJU, RIDE, and THOR was collected at

the Subtropical Livestock Research Institute, and the blood of KYRG collected at the Kyrgyzstan National Academy of Sciences. These blood samples were collected randomly and avoiding parent-offspring or sib pairs according to the pedigree information of each institution. Genomic DNA for genotyping was extracted from blood samples. The quantity and quality of the extracted DNA was evaluated using a microplate spectrophotometer (Epoch; BioTek Inst., USA). SNP genotyping was performed using an Illumina GGP Equine SNP chip (Illumina, San Diego, USA) [2]. This animal experiment was approved by the Animal Experimentation Ethics Committee of the National Institute of Animal Science.

2.2 Quality control of the genotypes

Identification and QC of raw data were confirmed and filtered using the Plink1.9 software package, and R software was used to

confirm individual information and remove redundant sample information. Of a total of 61,746 SNPs, 2,001 SNPs with a missing genotyping rate of 90% or higher, 1,294 SNPs with a hardy-weinberg equilibrium (HWE) of 0.001 or less, and 2,895 SNPs with a minor allele frequency (MAF) of 1% or less were filtered out, resulting in 231 of a total of 55,556 SNPs. The object was utilized for analysis. After QC, the genotyping rate was as high as 0.981, and there was no change in the number of samples, so a total of 229 sample information was used for genetic diversity information analysis (Table 1).

2.3 Analysis

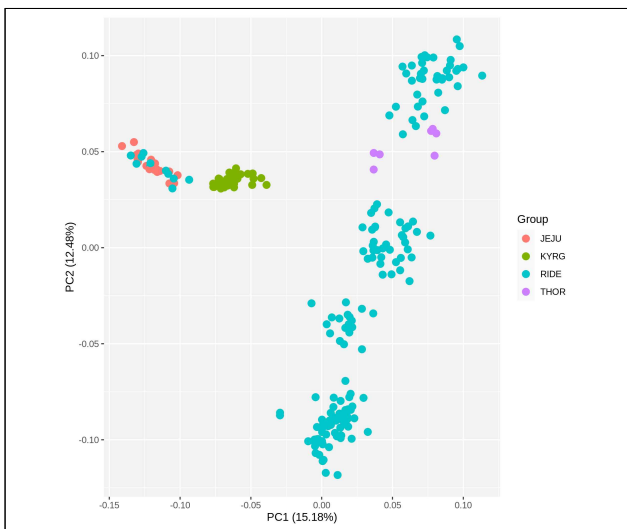
Multi-Dimensional Scatter (MDS) plot was used to confirm the clustering of the population using Plink1.9 software package, and Genetic distance and Phylogenetic tree were analyzed using R software.

[Table 1] Number of samples by breed

Breed	JEJU	RIDE	THOR	KYRG	Total
No. of Samples	24	166	8	31	229

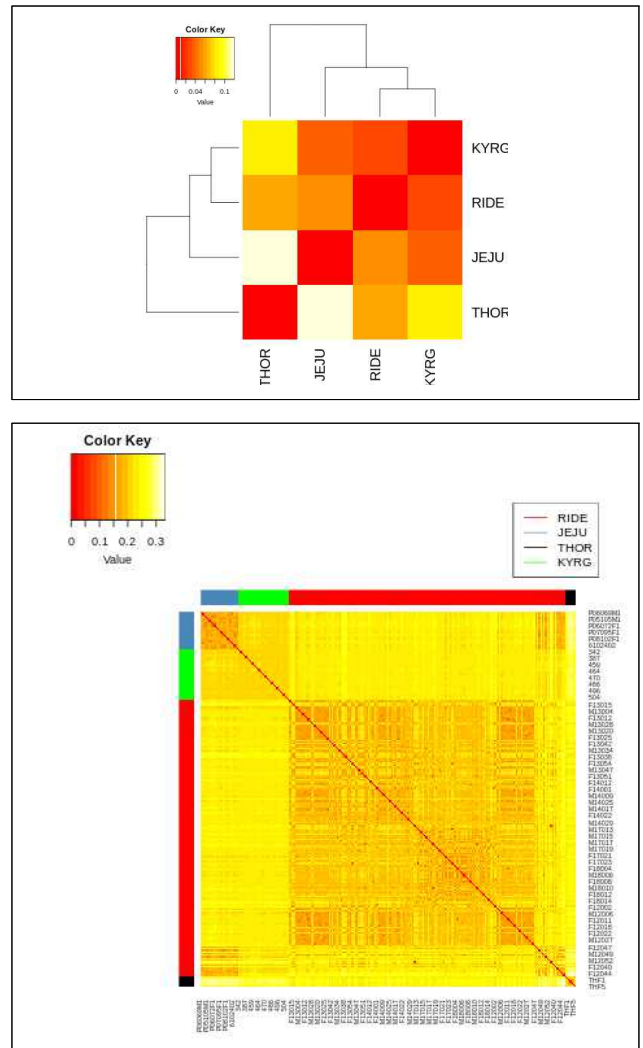
3. Results

As a result of confirming the cluster distribution of the population, it was confirmed that some RIDE clusters and JEJU breed were genetically related, and KYRG breed was able to confirm one cluster distribution (Fig. 1). In the case of the RIDE cluster on the right, genetic mixing of THOR and JEJU breeds was observed in the upper part[1].



[Fig. 1] MDS plot of two dimension by four horse breeds

In order to confirm the genetic distance for each breed, the allele frequency was calculated using the group SNP data for each breed, and the genetic distance information for each individual was extracted in the form of a diagonal matrix (Fig. 2). As for the genetic distance of the four groups, it was confirmed that THOR had a relatively far genetic distance compared to other groups, and that JEJU breed had a relatively closer genetic distance at some RIDE and KYRG breeds than THOR breed. As a result of checking the genetic distance of each individual, it was confirmed that the genetic distance between the RIDE breed was quite close, and the genetic distance between the KYRG breed was relatively far compared to other breeds. The KYRG breed, with high genetic diversity compared to other breeds, which is presumed to have a low degree of fixation and well-maintained genetic diversity.



[Fig. 2] Genetic distance heatmap by individual and breed

References

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- [2] Schaefer R. J. “Equine SNP Genotyping Arrays”, *Veterinary Clinics of North America : Equine Practice*, pp. 183-193, August 2020.