Genetic Diversity of Donkey (*Equus asinus*) by Mitochondrial DNA (mt-DNA) Analysis

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**Abstract**

Little research has been conducted on domestic donkeys compared with other livestock and companion animals in South Korea. There is no database that records the origins of domestic donkeys in South Korea, although most of the origins of Korean domestic donkeys are known to be in the northern areas (China, Mongolia). The present study conducted research to shed light on the phylogenetic characteristics and migratory route of domestic donkeys. This is the first mitochondrial DNA (mt-DNA) analysis of donkeys in South Korea. Mt-DNA base pairs of five Korean domestic donkeys were analyzed. A total of 16,670 base pairs and 38 related genes were observed in these donkeys. The result of an analysis of haplotype in CytB domain, revealed that there were 5 polymorphisms, 3 haplotypes, a haplotype diversity value of 0.800 and nucleotide diversity value of 0.00211 in domestic donkeys. As a result of analyzing the phylogenetic analysis based on these results, it is assumed that domestic donkeys were introduced to South Korea via China originated from the Somali wild ass breeds of northern region.

**Key words:** Donkey, mt-DNA, Haplotype, Phylogenetic Analysis, Somali wild ass, Korea

**Introduction**

Donkeys are one of the largest livestock that do not even study the most, although economically important. The process of domestication of the donkey is particularly interesting because the donkey has been widely used by humans a means of transport, are reported to have been domesticated approximately 5,000–6,000 years ago (Clutton-Brock 1992; Blenc 2004; Smith and Pearson 2005; Rossel et al. 2008; Davis 2019). The domestication of the donkey dramatically changed the ancient transportation systems in Africa and Asia, indicates a major cultural move away from settlement and an agrarian society life-style towards more relocation and trade (Beja-Pereira et al. 2004; Rosenbom et al. 2015).

There are approximately 1,000 donkeys bred in South Korea, mainly for tourists and meat, but there is little research on them, especially when it comes to their origins (Yun et al. 2018). The equine genome contains nearly 3,000 megabases (Mb) of DNA, similar to other mammals. The DNA is distributed in 31 autosomes as well as the X and Y heterosomes. A horse has 50,000 to 70,000 genes, which consist of repeated bases. These repeated bases have various forms such as minisatellite, microsatellite, α-satellite, SINE, and LINE. Repeated bases are very useful in genetic studies because they enable the differentiation of individual identification, parentage verification, gene mapping, and determination of breeds (Binns et al. 2000).

The horse mitochondrial genome is composed of approximately 16,660 nucleotides. Due to the strict maternal inheritance of mt-DNA, individuals within a maternal family line share the same mt-DNA haplotypes, thereby allowing the maternal line assignment accuracy to be evaluated (Wan et al. 2004). Several researchers have shown that using two or more mt-DNA markers might be more powerful for genetic diversity analysis (Pedrosa et al. 2005). The mt-DNA D-loop does not contain genetic information, which is usually utilized as a very useful utensil in phylogenetic analysis (Groeneveld et al. 2010; Han et al. 2014). Genetic studies based on mt-DNA pointed out that African wild donkeys are the most likely ancestors of domestic donkeys, but questions about their origins remain unresolved (Rosenbom et al. 2015).

In *Equus caballus*, the mt-DNA sequence was decided by Xu and Arnason (1994) and researched by Oakenfull et al. (2000) and Vilstrup et al. (2013). Analysis of mt-DNA
genome from a modern horse appeared that there were 18 haplogroups, and the various diverse genetics of wild horses were mixed into domestic species during the domestication process (Lippold et al. 2011; Achilli et al. 2012).

The Equidae family includes a single genus Equus which is classified into four subgenera having eight species. Donkeys are classified in the equine family, which also includes horses, zebras, and mules (Wilson 1990).

For centuries, donkeys have played an important role in agriculture and transport (Xie 1987; Seyiti and Kelimu 2021). Currently, donkeys are useful not only for meat, but also for Ejiao (a kind of Chinese medicine made by donkey skin), milk, and cosmetics manufacturing raw materials (Kumeta et al. 2014; Davis 2019). Also, donkeys are used as a working power in several world regions (Cozzi et al. 2018).

As a result of investigating the morphological characteristics of donkeys raised in South Korea, the ear length (17-28cm, average 23cm), body height (90-135cm, average 118.3cm), and body length (109-150cm, average 131.2cm) were confirmed. Coat colors was distributed white, chestnut, gray, black, and brown (Yun et al. 2018). Most of the domestic donkeys are believed to have come from northern areas such as China, but there is no accurate literature in South Korea. The present study was conducted to examine the phylogenetic characteristics and route of introduction of domestically bred donkeys.

MATERIALS AND METHODS

DNA Extraction, PCR Amplification and Sequencing

The mt-DNA was extracted from whole blood samples (3-5mL) of 5 donkeys using the QIAamp DNA Mini Kit (QIAGen, Hilden, Germany) according to the manufacturer's instructions. The DNA was quantified using NanoDrop™ 1000 Spectrophotometer. In order to analyze of mt-DNA sequence, two pairs of primer (F-CCCAAGGACTATCAA, R-GTTCTTTCTTCAGGGCCATT, GenBank X97337) were designed. For PCR, 2μL template DNA, 2μL of both 10pmol forward and reverse primers, and 2μL sterile distilled water were mixed in PCR Premix buffer (QIAGen, Hilden, Germany), adjusted to a total volume of 15μL, and then amplified by the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). The PCR amplification was as follows; initial denaturation for 5min at 94°C, followed by 35 cycles (denaturation at 94°C for 30s, annealing at various temperatures for 30s and extension at 72°C for 60-120s). An extension process at 72°C for 7 min was added after the final cycle.

PCR products were purified using Millpore plate MSNU030 (Millipore SAS, Molsheim, France), then Sanger-sequenced with the BigDye terminator v3.1 sequencing kit and a ABI 3730xl Automated Sequencer (Applied Biosystems, Foster City, USA).

Nucleotide sequences were determined on both strands of PCR amplification products at Macrogen Sequencing Facility (Macrogen Inc., Seoul, Korea). Nucleotide sequence data were analyzed with Variant Reporter Computer Software Version 1.1 (Applied Biosystems, Foster City, USA), BioEdit v7.2.5 (Hall 2004) Sequence Alignment Editor Software was used to prove and correct individual electropherograms of the sequences. All sequence alignments were performed using a general reference sequence (GenBank).

Data Analysis

DNA sequence polymorphism software (DNASP ver. 5.1) was used to explore the variation in regions, estimate hereditary variation, and determine the haplotypes (Librado and Rozas 2009). For an analysis of the cousin relationships of haplotypes and the hereditary relationship between groups, Network Software Ver. 4.5 was used (www.fluxus-technology.com) based on the result of donkey base sequence registered 70 world donkeys in the NCBI database (Rozas et al. 2003).

RESULTS

The results of the mt-DNA genome analysis are presented in Table 1 and Fig. 1. A total of 16,670 bases were observed in this study. Also, a total of 38 related genes were observed including 22 genes that indicate tRNA, 2 genes that indicate rRNA, 13 genes that provide information to produce proteins, and 1 control region.

The analysis of haplotypes in the cytB domain is shown in Table 2. Data on cytB in donkey stored in the NCBI revealed that the number of polymorphic sites was 89; the number of haploids, 68; the diversity of haploids, 0.997; and the diversity of acid, 0.00911. The result of an analysis of 5 domestic donkeys showed that the region of polymorphisms was 5; the number of haploids, 3; the diversity of haploids, 0.800; and nucleotide diversity, 0.00211, which was relatively less than that of donkeys registered with NCBI.

The result of a network analysis using the median joining method based on the 68 haplotypes observed is shown in Fig. 2. As shown in Fig. 2, the 68 haplotypes of 75 individuals including 5 domestic donkeys were classified into Clades A (left, Somali E. asinus), B (top center, Chinese E. asinus), C (bottom center, Chinese E. asinus), and D (right, domestic E. asinus) through 15 Median Vectors (MVs).

DISCUSSION

In contrast with genomic DNA, mt-DNA nucleotide sequences are a recognized utensil for resolving phylogenetic relationships at different evolutionary level of its special properties (Sun et al. 2016). Also, mt-DNA is very useful for relationship studies, which compare close species of animals and plants (Tanaka et al. 2014; Iorizzo et al. 2012).

In the present study, an analysis of the mt-DNA genome showed a total of 16,670 bases. This was shorter than the 16,813 bp reported by Sun et al. (2016), but showed similar results. Also, the results of cytB analysis of domestic donkeys presented relatively small value, indicating that there are few breeding individuals and little blending within the population of domestic donkeys in South Korea. Cozzi et al. (2018) studies 6 Italian donkeys breeds using mt-DNA D-loop, and found 15 haplotypes, grouped in three haplogroups and Xia et al. (2019) reported that the haplotype diversity of Egyptian donkeys and Brazilian donkeys was 0.910±0.032, 0.879±0.060, respectively, showing slight differences from the results of this study. Therefore, research on more donkey breeds samples is required in the future.
Due to modern industrialization, the number of donkeys has decreased significantly, though the ways they are used have greatly diversified (Xie 1987; Xu et al. 2012). Recently, there has been an increase in raising donkeys used as meat and a component of traditional Chinese medicine (Lei et al. 2007; Kumeta et al. 2014).

Donkeys raised in South Korea are mainly bred for tourism and meat, but systematic population management and disease management on donkey have been less than that on other animals. In Korea, donkeys are expected to get a lot of attention in the future as a high value meat product systems. To aim this purpose, it is necessary to develop and improve donkey breeding technology. Further research is also needed to manage the...

Fig. 1: Map of the donkey mitochondrial genome in South Korea.

Fig. 2: Median network profile of mt-DNA haplotypes observed in this study. Red circles are median vectors used in connecting in directly related haplotypes. Clades A (left, Somali E. asinus), B (top center, Chinese E. asinus), C (bottom center, Chinese E. asinus), and D (right, domestic E. asinus).

lineage of domestic donkeys. And, additional research is required to promote welfare, such as the management of donkey hooves and disease management.

Conclusion
Most of the donkeys raised in South Korea are known to have come from northern regions (China, Mongolia), but there is no accurate literature in Korea. As a result of this study, it was confirmed that these donkeys originated from the Somali wild donkey species among the Somali donkeys and the Nubian donkeys raised in China.

Author’s Contribution
All research protocols and animal experiments in this study designed, conducted experiments by SW Yun and contributed to data acquisition. GJ Cho contributed to the interpretation of the experimental results and the writing of the manuscript.

REFERENCES


