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African Maternal Origin and Genetic Diversity of Five Chinese Domestic Donkeys Breeds

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Abstract: To clarify the origin of Chinese domestic donkeys, the researchers investigated the partial mitochondrial D-loop sequences of 145 samples from 5 native breeds. The results revealed two mitochondrial origins. In the analysis of sequences of 5 native breeds between previously published sequences from other countries/regions and sequences of Chinese domestic donkeys, the results indicated that the two lineages of Chinese domestic donkeys were from Africa and supported the African maternal origins of Chinese domestic donkeys. The population showed abundant mtDNA diversity.

Key words: Chinese domestic donkeys, MtDNA, D-loop, origin, medicine, China

INTRODUCTION

China has the largest donkey (*Equus asinus*) population in the world, owning about 4000 years history of raising donkeys. Donkeys are widely distributed throughout 17 provinces of the Central, Northeastern and Western China, primarily in Western China around the Huanghe valley (Xie, 1986). The origin and evolution of Chinese domestic donkeys (*Equus asinus*) is still uncertain. Although, previous genetic studies revealed two mitochondrial DNA (mtDNA) types in a few Chinese donkey breeds.

Mitochonarial DNA (mtDNA) encodes for 13 proteins, 2 ribosomal genes and 22 tRNAs. Meanwhile mitochondria use oxidative phosphorylation to convert dietary calories into energy in the form of ATP and heat that is essential in the life of mammalian cells. The abundance of mitochondrial DNA polymorphism has made it one of the most popular markers used in population genetic studies (Avise, 2000). In previous studies, various studies engaged in livestocks have been tried to identify associations between mtDNA polymorphisms and important economical traits (Ivankovic *et al*., 2002; Jeon *et al*., 2005; Odahara *et al*., 2006; Sasazaki *et al*., 2006; Liu *et al*., 2007). Here in the researchers sequenced a 399 bp fragment of the mtDNA Control Region (CR) of 145 individuals from five Chinese donkey breeds and compared the sequences with published sequences from donkeys from the old world so as to understand the origin of Chinese donkeys.

MATERIALS AND METHODS

Sample collection and DNA extraction: A total of 145 fresh blood samples were collected from 5 native domestic donkey breeds in China and stored at -80°C. The genomic DNA was extracted from blood by standardphenol-chloroform method. In addition, three African Somali wild donkeys (AY569545-AY569547), three croatia domestic donkey (AF403063-AF403065), six Asian wild donkeys including *E. kiang* (AF220932-AF220933), *E. hemionus kulan* (AF220934-AF220936) and *E. hemionus onager* (AF220937) in GenBank were collected in this study. The distributions of all 5 Chinese domestic donkey breeds were shown in Fig. 1. Geographic location and number of donkey samples were shown in Table 1.

PCR amplification and sequencing: To amplify the partial D-loop region of donkey mtDNA, a pair of primers was synthesized according to the published sequences, DA: 5'-AGTCTCACCATCAACACCCAAAGC-3' and DB: 5'-CCTGAAGTAGGAACCAGATG-3' (Ivankovic *et al*., 2002). PCR amplifications were conducted in a 50 μL volume containing 5 μL of 10×buffer (with Mg^{2+}), 0.25 mM dNTPs, 0.2 μM each primer, 2.5 U Taq DNA polymerase (TaKaRa Biosystems) and approximately 20 ng genomic DNA. The PCR was carried out using a standard program with 4 min denaturation at 95°C, 35 cycles for 45 sec at 94°C, 60 sec at 56°C and 90 sec at 72°C and final extension for 10 min at 72°C. PCR products were purified with Watson PCR Purification Kit (Watson

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Table 1: Source of the donkey samples in China

Breeds (Abbreviations)	Geographic distribution	Samples
Guanzhong (GZ)	Fufeng county, Shaanxi province	41
Dezhou (DZ)	Wuli county, Shandong province	25
Jinnan (JN)	Xia county, Shaanxi province	30
Guangling (GL)	Shouyang county, Shaanxi province	24
Bohai (BH)	Wuli county, Shandong province	25

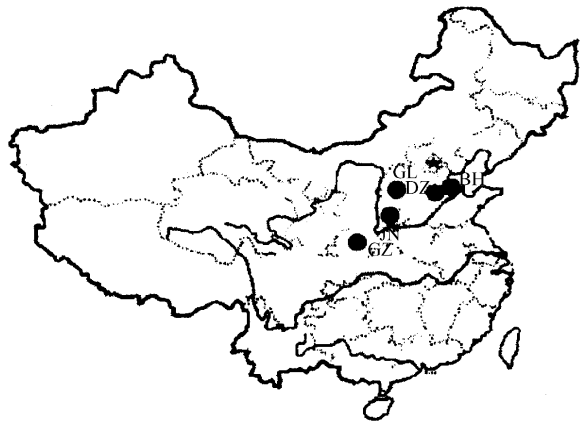

Fig. 1: Geographical distribution of five Chinese donkey breeds sampled

BioTechnologies, Shanghai). Sequencing was performed by using an ABI model 3730 automated sequencer.

Data analysis: Sequences were edited by DNASTAR5.0 package (DNASTAR, Madison, WI). All sequences of mtDNA D loop were aligned in the ClustalX package (Thompson *et al*., 1997).

The polymorphisms in the analyzed segments and the pairwise mismatch distribution between donkey sequences were obtained using the Arlequin 2.0 computer package (Schneider *et al*., 2000). The polymorphisms in the analyzed segments were exported by using MEGA2.1 (Kumar *et al*., 2001). A Neighbour-Joining (NJ) tree using Kimura-2-parameter model with 1,000 boot strapping replicates was constructed based on the aligned sequencesto identify possible phylogenetic lineages in MEGA 2.1 (Kumar *et al*., 2001). Reduced median networks (Bandlet *et al*., 1999) were generated using the Network 4.1 program. The haplotype diversity (h), nucleotide diversity (π) for the breeds were estimated by using DnaSP 4.0.

RESULTS AND DISCUSSION

MtDNA variation and haplotype: Comparison of the 145 mtDNA D-loop sequences of 399 bp in five Chinese domestic donkeys breeds revealed 37 different haplotypes with 32 polymorphic sites (Fig. 2). There were no insertions/deletions observed in 145 mtDNA D-loop sequences of 399 bp in five Chinese domestic donkeys breeds of these polymorphic sites, there were 30

Table 2: Genetic diversity indices in 5 Chinese donkey breeds

Code of breed	Sample size	Haplotype diversity	Nucleotide diversity	Mean number of pairwise different
GZ	41	0.888±0.028	0.02265±0.00074	9.039±3.164
DE	25	0.920±0.033	0.02386±0.00188	9.520±3.438
JN	30	0.880±0.036	0.02162±0.00207	8.628±3.074
GL	24	0.902±0.037	0.02287±0.00152	9.127±3.307
BH	25	0.897±0.043	0.02047±0.00245	8.167±2.949
Avg	145	0.917±0.011	0.02265±0.00040	9.039±3.055

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[  4569344556 6889001245 5678366678 88]
[  6255112240 1230674401 7070234882 34]                N
H1  ATCAGCGAAT GTAAAGTGCC CTACCTTCTT AA                15
H2  ..........A....A.... .....T.. ..                    4
H3  GCT.A.A..C ....GACA... .C..TC...C .G               16
H4  .......... .....A.... .....T.. .                   25
H5  .......... .....A.... .....CT.. ..                  1
H6  GCT.A.A..C ....GACA... .C..TC...C GG                24
H7  .....A.... ..G..A.... .....T.. ..                   5
H8  ..........CG..A.... .....T.. ..                     5
H9  .....A.... .....A.... .....T.. ..                   1
H10 .......... ..G..A.... .....T.. ..                   5
H11 ..T.A.A..C ....GACA... .C..T.T.C ..                 1
H12 .......... ..G..... .....T.. ..                     1
H13 GCT.A.A..C ....ACA... TC..TC...C GG                 5
H14 GCTGATA..C ....GACAT. ....TC...C GG                 2
H15 GCT.A.A..C ....GACA... .CT.TC...C .G                4
H16 GCT.ATA..C ....GACAT. .C..TC...C G G                5
H17 .......... .CG..A... T .....T.. ..                  1
H18 GCT.A.A..C ....ACA... .C..TC...C GG                 1
H19 ..T.A.A..C .....ACA.. TC..TC...C GG                 1
H20 .......... .....A.... ....C.T.. ..                  1
H21 .......... A...GA.... .....T.. .                    1
H22 .....A.... .......... .......... ..                 2
H23 .....A.... A....A.... ..C.....T.. ..                1
H24 GCT.A.A..C ....GACA... .C..T....C .G                2
H25 .......... ....GA.... .....T.. ..                   2
H26 .....A.... ....G.A.... ....T.C.T.. ..               1
H27 .....A.... ...G..A.... .....C.T.. ..                1
H28 .......... ..G..A.... .....TC.. ..                  1
H29 ..T..... .....A.... .....T.. ..                     1
H30 GCT.A.A..C A...GACA... .C..TC...C GG                2
H31 GCT.A.A.TC ....GACA... .CT.TC...C .G                1
H32 GCT.A.A..C ....GAC.... .C..TC...C GG                2
H33 .......... .......... .....T.. ..                   1
H34 .....A.... ..GG.A.A.. .....C.T.. ..                 1
H35 .....G.... .....A.... .....T.. ..                   1
H36 .....A.... A....A.... .....T.. .                    1
H37 .......... A....A.... ....TC.T. ..                  1
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Fig. 2: MtDNA D-loop sequence variations detected in 37 haplotype of five Chinese donkey breeds, N represents sample size

transitions and 2 transversion suggesting the strong bias towards transitions. Of the 37 haplotypes, there are 20 unique haplotypes and 17 shared haplotypes among five donkey breeds. The most popular haplotype is Hap_4 consisting of 25 samples from 5 breeds followed by three haplotype (Hap_6, Hap_1, Hap_3), each consisting of ≥15 samples. The haplotypes Hap_8, Hap_13, Hap_7, Hap_10 and Hap_16 are less popular, each consisting of ≥5 samples (Fig. 2). The haplotype number in each breed varies from 11-14 and haplotype diversity values differ from 0.888±0.028 in the Jinnan to 0.920±0.033 in the Dezhou breed. Nucleotide diversity values range from 0.02047±0.00245 in the Bohai to 0.02386±0.00188 in the Dezhou breed. Mean value of pairwise differences varies from 8.167±2.949 in the Bohai to 9.520±3.438 in the Dezhou donkey (Table 2). The haplotype and nucleotide diversity values within Chinese donkeys were 0.917±0.011 and 0.02265±0.00040, respectively indicating abundant genetic diversity. The level of genetic diversity

among Chinese donkey population is higher than that of Spain (Nucleotide diversity, π = 0.007) (Aranguren-Mendez *et al*., 2004).

Phylogenetic tree construction: The researchers constructed a NJ tree and a UPGMA tree with 37 mtDNA haplotypes from 145 Chinese donkeys, 3 croatia donkey sequences, 3 Somali wild donkey and 6 Asian wild donkey sequences. This phylogenetic tree clearly demonstrates that there are two divergent maternal lineages existed in Chinese donkey population (Fig. 3). The two lineages included 18 and 25 haplotypes representing 70 and 81 samples, respectively. The new results clearly exclude the Asian wild donkey as progenitors of Chinese domestic donkeys and African wild donkeys are the likely progenitors of Chinese domestic donkeys (Fig. 3). Therefore, the results further support the viewpoint of origins of the domestic donkey (Beja-Pereira *et al*., 2004). Thirty-seven haplotypes detected in 145 donkeys suggest that there is abundant genetic diversity in Chinese donkey population. As shown in phylogenetic tree, the network also clearly revealed two lineages.

Population structure: Almost no geographic were detected in five Chinese donkey breeds (Fig. 1). These results indicated that there was no correspondence between the geographic among Chinese donkey breeds. This study presents the first substantial analysis of mtDNA diversity in Chinese donkeys and provides

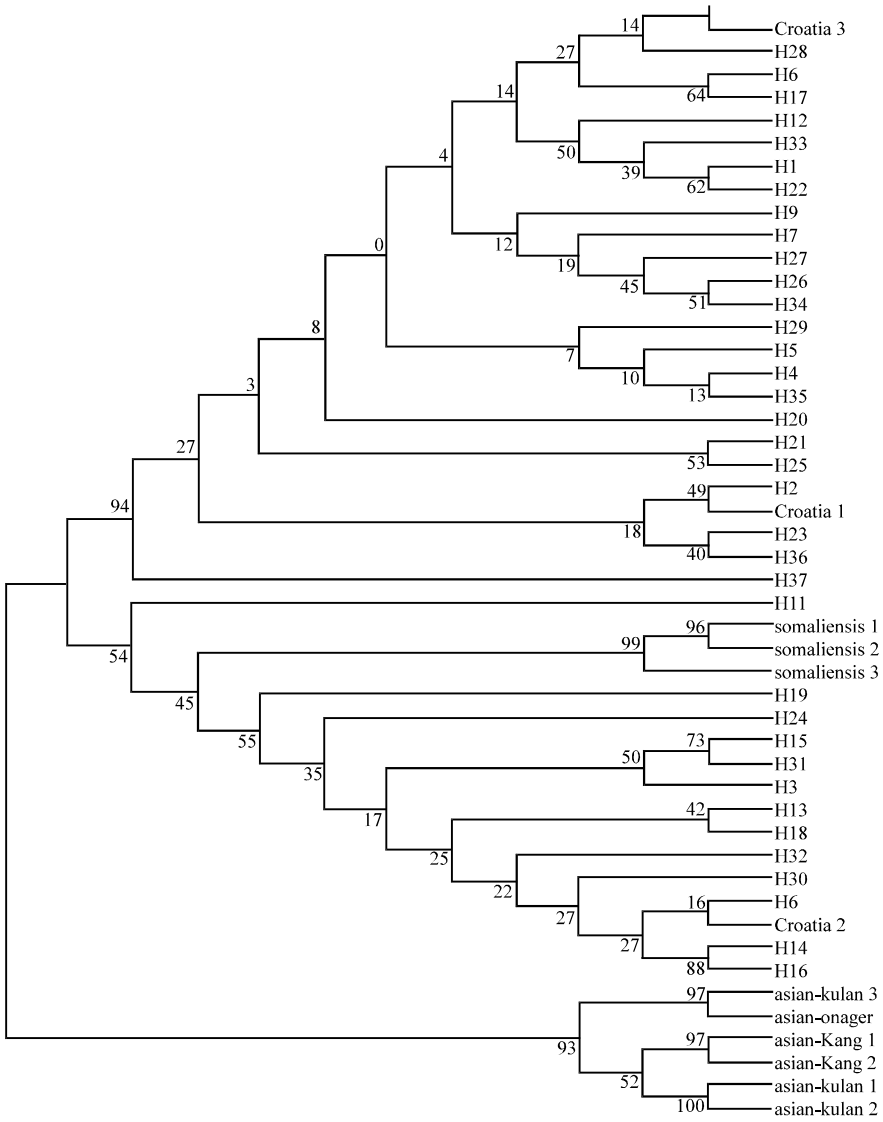

Fig. 3: Continue

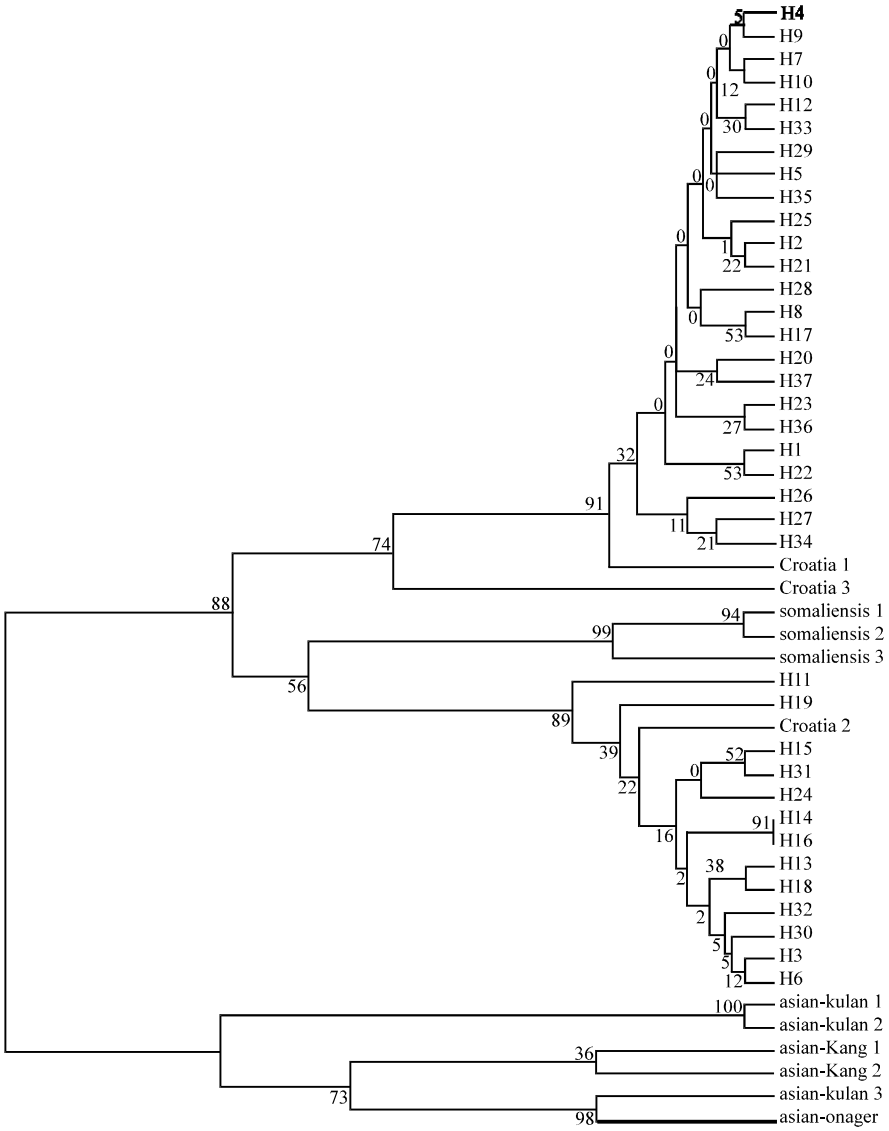

Fig. 3: NJ, UPGMA tree of five Chinese donkey breeds, Asian wild ass, somliensis wild ass and Croatia donkey

information about the origins and genetic structure of donkey breeds and thus insights into their genetic history and migration routes. About the origin of Chinese domestic donkeys, two hypotheses were proposed. Firstly, Chinese domestic donkeys originated from African wild ass; secondly, Chinese domestic donkeys originated from African wild ass and Asian wild ass (Xie, 1986). On one hand, Xinjiang region is adjacent to Iran, Afghanistan which are the domestication center of Asian wild ass, Qinghai, Tibet and Inner Mongolian region in China where are the important distribution areas of Asian wild ass. On the other hand, Chinese domestic donkeys are similar to Asian wild ass in the coat colour and outward morphologic features (Xie, 1986). However, in the study, two highly divergent mtDNA lineages of evidence support the first hypothesis that Chinese domestic donkeys originate from African wild ass, exclude the Asian wild ass as ancestors of Chinese domestic donkeys (Fig. 3). The analysis revealed abundant genetic diversity within breeds and among breeds. The rich genetic diversity existing in five Chinese donkey breeds is favorable to preserve the precious genetic resources.

CONCLUSION

This study demonstrated abundant mtDNA diversity existing in Chinese domestic donkeys. No obvious geographical structure was found among Chinese donkey breeds. The maternal origins of Chinese donkeys most likely derived from Africa.

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