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African Maternal Origin and Genetic Diversity of Chinese Domestic Donkeys

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ABSTRACT : The origin of domestic donkeys in China has been controversial. To clarify the origin of Chinese domestic donkeys, we investigated the partial mitochondrial D-loop sequences of 126 samples from 12 native breeds. The results revealed two mitochondrial origins, lineage Somali and lineage Nubian of African wild ass detected in Chinese domestic donkeys. Lineage Somali was predominant in Chinese domestic donkey breeds. The pattern of genetic variation in ass mtDNA D-loop sequences indicated that the two lineages Somali and Nubian from China had undergone population expansion events. In a combined analysis of lineages Somali and Nubian 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. There was no obvious geographical structure in Chinese domestic donkey breeds, but the population showed abundant mtDNA diversity. The spread routes of Chinese domestic donkeys were also discussed. (**Key Words** : Chinese Domestic Donkeys, MtDNA D-loop, Origin)

INTRODUCTION

To date, little is known about the origin and genetic diversity of domestic donkeys in China. This might partly be due to the lower performance of donkeys with respect to economic important traits when compared with cattle, pigs, goats, sheep and chickens, thus limiting the interests in genetic analysis of donkeys in China. However, China is one of the countries especially rich in donkey genetic resources in the world. Donkeys are widespread throughout 17 provinces of the Central, Northeastern and Western China, primarily in Western China around Huanghe valley with dry, arid, semi-arid and warm climate, but are also found in the cold climate in three provinces of Northeastern China (Xie, 1987). With the opening to the outside world in 1978, China has changed everywhere. In the past decades, unfortunately, donkey population rapidly decreased in many regions of China, particularly the regions of fast developed. Many breeds, such as famous Guanzhong donkey, are in the conservation status for their genetic resources. However, in less developed regions, donkeys are still the important domestic animals for transport, meat and Chinese medicine ingredients. However, in recent years, the demand for donkey meat is rapidly increasing. Since its high nutrition value, donkey meat has been considered as a green food. The market demand for donkey meat makes more farmers raise donkeys for commercial purpose.

Mitochondrial DNA (mtDNA) D-loop (control region) sequences have been extensively used to investigate the origin and diversification of modern domestic donkey, cattle and goats populations (Ivankovic et al., 2002; Jeon et al., 2005; Odahara et al., 2006; Sasazaki et al., 2006; Liu et al., 2007). For instance, Ivankovic et al. (2002) were the first to investigate the mtDNA D-loop genetic diversity of Croatial donkey populations and found three haplotype groups, which constituted two primitive maternal lineages. Lopez et al. (2005) analysed the genetic origin and genetic diversity of the Mexican Creole donkeys, and showed an African origin. Aranguren-Mendez et al. (2004) studied the mtDNA genetic diversity of six Spanish donkey breeds and two African donkey populations and confirmed two divergent maternal lineages of African origin (*E. a. Africanus* and *E. a. Somaliensis*). Beja-Pereira et al. (2004) carried out a

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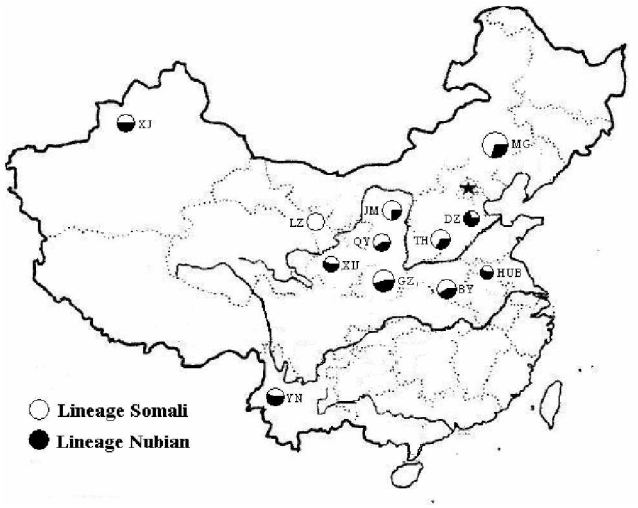

Figure 1. Geographical distribution of the Chinese donkey breeds sampled. Circle area is proportional to sample sizes. The abbreviations of 12 Chinese donkey breeds were shown in Table 1.

worldwide survey of domestic donkey mtDNA D-loop (479 bp) diversity and identified two highly divergent mtDNA lineages. The two lineages were estimated to have diverged over 0.303-0.910 million years ago by analyzing complete cytochrome b sequences of domestic donkey; this ancient divergence time suggested two separate maternal origins of domestic donkey from two distinct wild populations (Nubian and Somali). Few reports are available, however, on mtDNA D-loop sequence variation and origin of domestic donkeys in China (Lei et al., 2005). The previous analysis of Chinese donkeys was confined to a single study by using a limited 10 and 26 samples, respectively (Beja-Pereira et al., 2004; Lei et al., 2005). There have been two hypotheses about the origin of Chinese domestic donkeys. Firstly, Chinese domestic donkeys might have originated from African wild ass. Secondly, Chinese domestic donkeys originated from both African wild ass and Asian wild ass (Xie, 1987). In this study, we sequenced partial mitochondria D-loop sequences of 12 Chinese domestic donkey breeds and provided some evidences for their evolution, origin, genetic diversity and spread routes.

MATERIALS AND METHODS

Sample collection and DNA extraction

126 fresh blood samples were collected from 12 native domestic donkey breeds in China and stored at -70°C. The genomic DNA was extracted from blood by standard phenol-chloroform method. In addition, 79 haplotypes all over the world (AY569462-AY569538, AY569543-AY569544) and three African Somali wild donkeys (AY569545-AY569547), three Nubian wild donkeys (kindly provided by Dr. Albano Beja-Pereira) and 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 12 Chinese domestic donkey breeds were shown in Figure 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'-AGTCTCACCATCAACACC CAAAGC-3' and DB: 5'-CCTGAAGTAGGAACCAGA TG-3' (Ivankovic et al., 2002). PCR amplifications were conducted in a 50 μl volume containing 5 μl of 10×buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.2 μM each primer, 1.5 U *Taq* DNA polymerase (TaKaRa Biosystems), and approximately 50 ng genomic DNA. The PCR was carried out using a standard program with 4 min denaturation at 95°C, 35 cycles for 30 s at 94°C, 60 s at 55°C, and 90 s at 72°C, and final extension for 10 min at 72°C. PCR products were purified with Watson PCR Purification Kit (Watson BioTechnologies, Shanghai). Sequencing was performed by using an ABI model 377 automated sequencer (PE company).

Data analysis

Sequences were edited by DNASTAR5.0 package (DNASTAR, Madison, WI). All sequences of mtDNA D-

Table 1. Source of the donkey samples in China

Breeds (abbreviations)	Geographic distribution	Samples	Type
Guanzhong (GZ)	Fufeng county, Shaanxi Province	13	Large
Dezhou (DZ)	Dezhou city, Shandong Province	7	
Qingyang (QY)	Qingyang city, Gansu Province	10	Medium
Jiami (JM)	Mizhi county, Shaanxi Province	11	
Beiyang (BY)	Beiyang county, Henan Province	12	
Mongolia (MG)	Kulun county, Inner Mongolian Autonomous Region	20	Small
Xiji (XIJ)	Xiji county, Ningxia Autonomous Region	7	
Huaibei (HUB)	Huaibei city, Anhui Province	5	
Liangzhou (LZ)	Wuwei city, Gansu Province	10	
Taihang (TH)	Linzhou city, Henan Province	12	
Xinjiang (XJ)	Yining city, Xinjiang Autonomous Region	10	
Yunnan (YN)	Chuxiong city, Yunnan Province	9	

[	1111111111	11111111111111111111	1111111	
[	5555555555	5555555555	5555555555	5555555]	
[	4445555555	5666666666	6666677777	7888888]	
[	7880046899	9222234456	7799901117	7000222]	
[	6460319078	9016874522	4805843580	7126012]	N
TH20	AAATCTGATT	GTAAACAATG	CCACTCCACC	TTCTTAA	1
XJ15	.....A.....	.T.....	.....	1
CD9	.....A.....	.....	..T....	3
BY31	.....G..A....T....	T.....	..T....	1
CD8	.....G..A.....	.....	..T....	4
CD10	.....G.....	.....	.....	3
CD5	.....G.....	.....	..T....	10
CD13	.....G...G.....	.....	..T....	2
JM35	.....G...G.....	.....	C.T....	1
XIJ9	.....G...G.....	.....T....	..T..G.	1
CD11	.....G..CG.....	.....	..T....	2
HUB23	.....G..CG.....	.....	.CT....	1
GZ975	.....G.....	.....	..T....	1
XJ17	.....G.G.....	.....T.	.CT....	1
YN18	.....G....G...G..	.....	..TC...	1
YN23	.....G.....G..	.....T....	..TC...	1
CD3	.....G.....G..	.....	..TC...	11
BY39	..G....G.....G..	.....	..TC...	1
QY1	.....G.....G..	.....	..T....	1
CD12	.....G...G....G..	.....	..T....	2
MG16	.....G.....	.....	.CT....	1
MG3	.G.CT.A..C .....G.CA	..G.C....T	.CTCCGG	1
XJ13	.G.CTCA..C .....G.CA	....C....T	.CTCCGG	1
CD1	.G.CT.A..C .....G.CA	....C....T	.CTCCGG	32
QY10	GGGCT.A..C .....G.CA	....C....T	.CTCCGG	1
QY11	GG.CT.A..C .....G.CA	....C....T	.CTCCGG	1
XJ14	.G.CT.A.CC .....G.CA	....C....T	.CTCCGG	1
TH6	.G.CT.A.CC .....G.CA	....C....T	.CTCC.G	1
CD14	.G.CT.A..C .....G.CA	....C...TT	.CTCC.G	2
YN2	.G.CT.A..C .....G.CA	....C...TT	.CTCCGG	1
CD2	.G.CT.A..C .....G.CA	....C....T	.CTCC.G	13
CD6	.G.CT.A..C .....G.CA	....C..T.T	.CTCC.G	6
XJ16	.G.CT.AG.C .....G.CA	....C....T	.CTCCGG	1
CD7	.G.CT.A..C .....G.CA	....C....T	..TCC.G	5
CD4	.G.CT.A..C .....CA	...TC....T	.CTCCGG	8
CD15	.G.CT.A... .....CA	...TC....T	.CTCCGG	2

Figure 2. MtDNA D-loop sequence variations detected in 36 haplotypes of 126 Chinese donkeys. N referred to the number of samples.

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 bootstrapping replicates was constructed based on the aligned sequences to identify possible phylogenetic lineages in MEGA 2.1 (Kumar et al., 2001). Reduced median networks (Bandelt et al., 1999) were generated using the NETWORK 4.1 program. The haplotype diversity (h), nucleotide diversity (π) and mismatch analysis for the breeds were estimated by using Arlequin 2.0 package (Schneider et al., 2000). All sequences are deposited in GenBank (Accession Nos. AF531459- AF531470, AF532118-AF532126, AY666165-AY666169, DQ368497-DQ368596).

RESULTS

MtDNA variation and haplotype

Comparison of the 126 mtDNA D-loop sequences of 399 bp in Chinese domestic donkeys revealed 36 different

Table 2. Genetic diversity indices in 12 Chinese donkey breeds

Code of breed	Samples	Lineage Somali		Lineage Nubian		Haplotype diversity	Nucleotide diversity	Mean No. of pairwise differences
		Haplotype	Samples	Haplotype	Samples			
GZ	13	2	7	4	6	0.8462±0.0649	0.0233±0.0129	9.2851±4.5650
DZ	7	1	2	3	5	0.8095±0.1298	0.0201±0.0122	8.0019±4.2360
MG	20	6	14	4	6	0.9158±0.0395	0.0210±0.0113	8.3747±4.0475
XIJ	7	3	3	3	4	0.9524±0.0955	0.0277±0.0164	11.0309±5.7170
QY	10	5	6	3	4	0.9556±0.0594	0.0256±0.0145	10.2212±5.1035
JM	11	4	8	3	3	0.8182±0.1191	0.0214±0.0121	8.5536±4.2856
BY	12	3	7	4	5	0.8333±0.1002	0.0262±0.0145	10.4663±5.1364
HUB	5	1	2	2	3	0.8000±0.1640	0.0278±0.0178	11.0863±6.0853
LZ	10	2	10	0	0	0.4667±0.1318	0.0012±0.0013	0.4675±0.4434
TH	12	5	8	2	4	0.9091±0.0562	0.0212±0.0119	8.4723±4.2178
XJ	10	5	5	4	5	0.9778±0.0540	0.0285±0.0160	11.3518±5.6327
YN	9	3	4	5	5	0.9722±0.0640	0.0272±0.0155	10.8420±5.4522

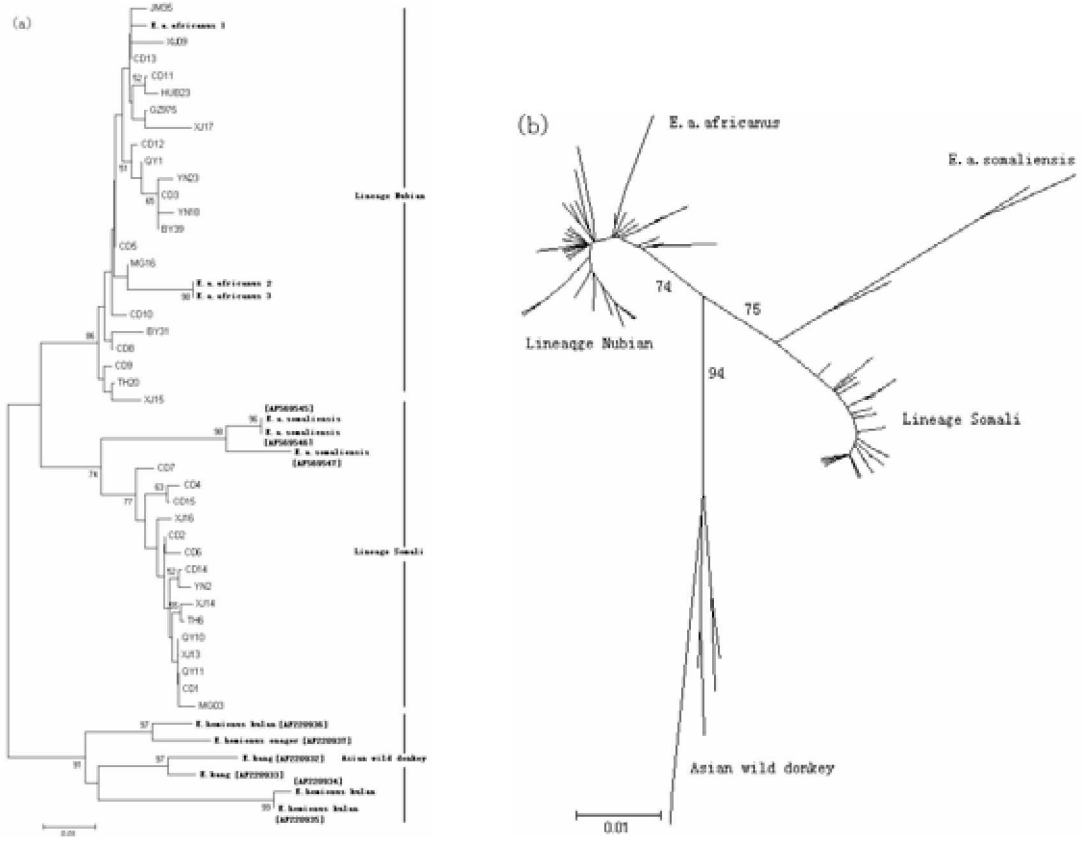

Figure 3. (a) The NJ tree of 36 mtDNA D-loop haplotypes of Chinese donkey, three Somali, three Nubian and six Asian wild donkey sequences. (b) The NJ tree of 36 Chinese mtDNA D-loop haplotypes and 79 haplotypes all over the world, three Somali, three Nubian and six Asian wild donkey sequences. Numbers at the two lineages denote the bootstrap percentages of 1,000 replications (only those ≥50% are shown).

haplotypes with 37 polymorphic sites (Figure 2). There were no insertions/deletions observed in 126 mtDNA D-loop sequences of 399 bp in Chinese domestic donkeys. Of these polymorphic sites, there were 36 transitions and one transversion, suggesting the strong bias towards transitions. Of the 36 haplotypes, there are 21 unique haplotypes and 12 shared haplotypes among donkey breeds. The most popular haplotype is CD1 consisting of 32 samples from 10 breeds except Xinjiang and Dezhou, followed by three haplotypes (CD2, CD3, CD5), each consisting of ≥10 samples. The haplotypes CD4, CD6 and CD7 are less popular, each consisting of ≥5 samples (Figure 2). The haplotype number in each breed varies from 2 to 10, and haplotype diversity values differ from 0.4667±0.1318 in the Liangzhou to 0.9778±0.0540 in the Xinjiang breed. Nucleotide diversity values range from 0.0012±0.0013 in the Liangzhou to

0.0285±0.0160 in the Xinjiang donkey. Mean value of pairwise differences varies from 0.4675±0.4434 in the Liangzhou to 11.3518±5.6327 in the Xinjiang donkey (Table 2). The haplotype and nucleotide diversity values within Chinese donkeys were 0.9055±0.0170 and 0.0228± 0.0117, respectively, indicating abundant genetic diversity. The level of genetic diversity among Chinese donkey population is higher than that of Spain (nucleotide diversity, $\pi = 0.007$) (Aranguren-Mendez et al., 2004).

Phylogenetic tree construction

To compare the haplotypes of Chinese donkey with previously nominated lineages Somali and Nubian (Beja-Pereira et al., 2004), we constructed an unrooted NJ tree with 36 mtDNA haplotypes from 126 Chinese donkeys and 12 African and Asian wild donkey sequences. This phylogenetic tree clearly demonstrates that there are two distinct mtDNA lineages Somali and Nubian existed in Chinese donkey population (Figure 3). The lineages Somali and Nubian included 15 and 21 haplotypes representing 76 and 50 samples, respectively. Our new results clearly exclude the Asian wild donkey as progenitors of Chinese domestic donkeys, and two African wild donkeys (Somali and Nubian) are the likely progenitors of Chinese domestic donkeys (Figure 3a). Further analyzing our 36 haplotypes and published 79 haplotypes all over the world also revealed two main lineages Somali and Nubian (Figure 3b). Therefore, our results further support the viewpoint of origins of the domestic donkey (Beja-Pereira et al., 2004).

The reduced median network (Figure 4) showed that both lineage Somali and Nubian had genetic contribution to the Chinese donkey evolution, as 15 haplotypes of lineage Somali and 21 haplotypes of lineage Nubian were identified in Chinese donkey population. Thirty-six haplotypes detected in 126 donkeys suggest that there is abundant genetic diversity in Chinese donkey population. As shown in phylogenetic tree, the network also clearly revealed two lineages and showed star-like phylogenetic pattern, in which two large haplotypes CD1 and CD5 located in the center of lineages Somali and Nubian, respectively (Figure 4 a and b). Lineage Somali was predominant (60.32%, 76/126), which was found in all 12 Chinese donkey breeds, and lineage Nubian was lower (39.68%, 50/126) (Table 2). To further analyze the detailed relationship, we combined

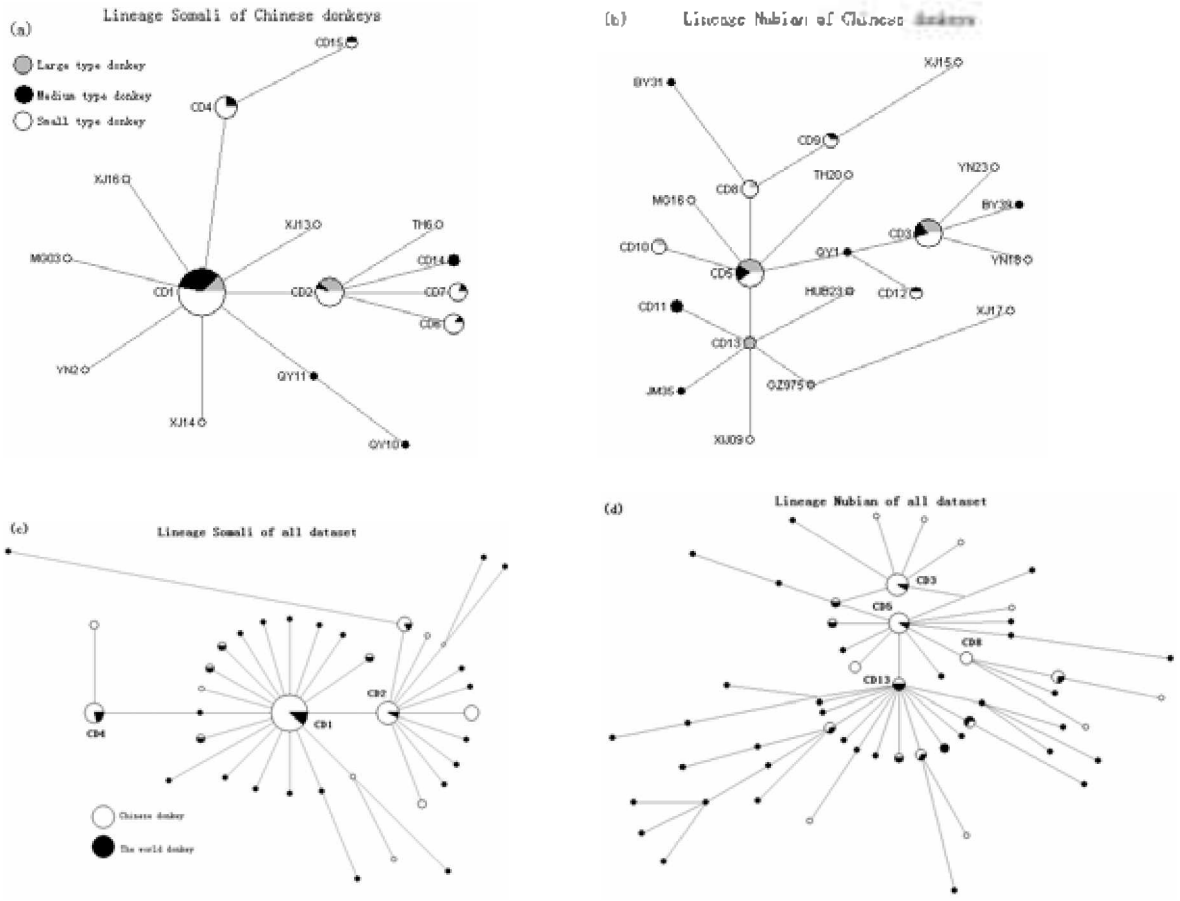

Figure 4. Reduced Median network of lineage Somali, Nubian for China (a, b) and all dataset of China, the world donkey sequences (c, d). The area of circle is proportional to haplotype frequency.

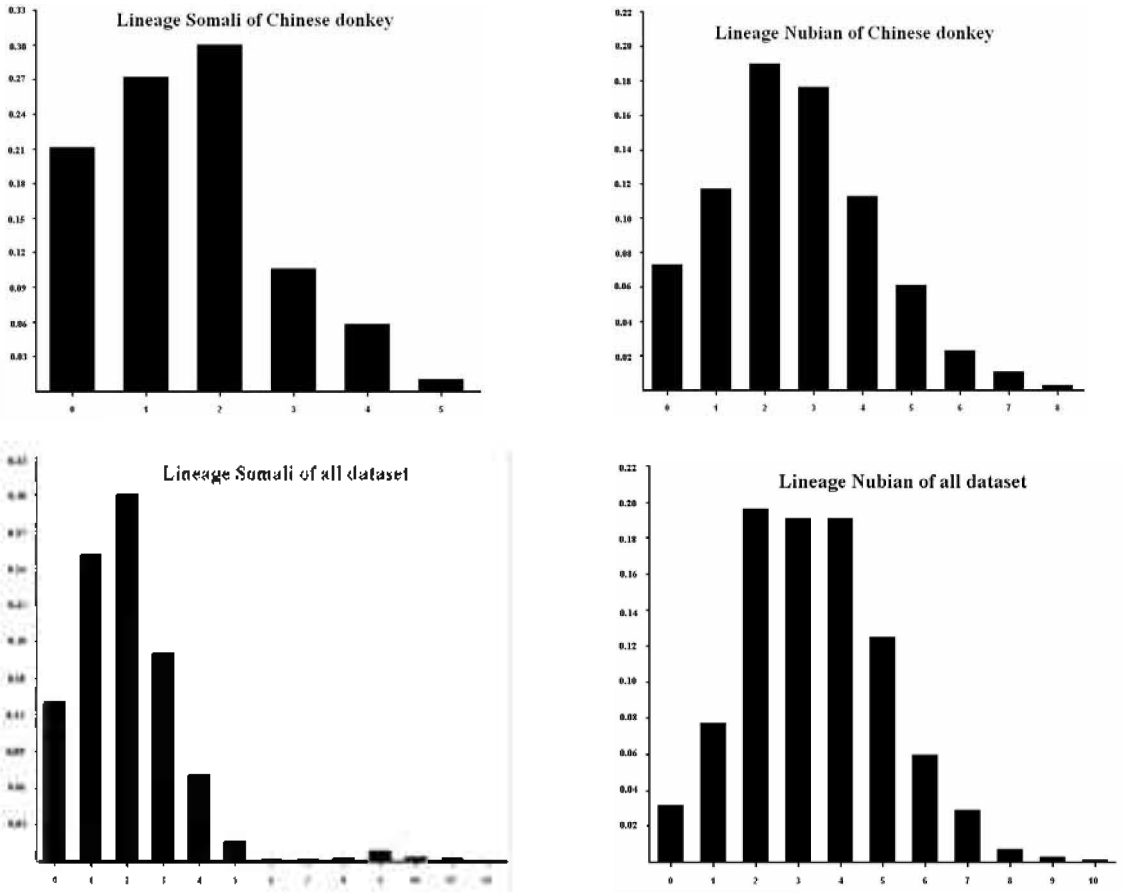

Figure 5. Mismatch distributions of mtDNA types of Chinese donkeys and reported data of donkeys.

all previously published 79 haplotypes of the mtDNA lineage Somali and lineage Nubian, respectively, with 36 haplotypes of Chinese donkey in our data. The analysis revealed 36 lineage Somali haplotypes and 54 lineage Nubian haplotypes, and the phylogenetic relationships among these haplotypes are shown in a reduced median network (Figure 4 c and d). The network also showed star-like phylogeny, in which two large haplotypes CD1 and CD5 located in the center of lineage Somali and lineage Nubian, respectively. MtDNA lineages in Chinese donkeys appear mixed with those of donkeys from the rest of the world. These results further supported the viewpoint that the Chinese domestic donkeys originated from Africa.

Population structure

Almost no geographic and body size structures were detected based on lineages Somali and Nubian in Chinese donkey (Figure 1). The donkeys of large, medium and small type belong to the mixture of lineages Somali and Nubian except LZ breed (Tables 1 and 2). The reduced median network (Figure 4 a, b) of 36 haplotypes in Chinese donkey showed the same results. These results indicated that there was no correspondence between the geographic regions, body types and origin among Chinese donkey breeds.

Population expansions

Historical demography of population appeared to have had profound effect on patterns of genetic variation of mtDNA and genetic diversity (Donnelly et al., 1996). Mismatch distributions of mtDNA have been widely used to explore demographic history of populations. Analyses of mismatch distributions of mtDNA for Chinese donkeys revealed the bell-shaped curve and star-shaped phylogenies (Figure 5), which were consistent with a demographic population expansion. The approximately relative dates of expansion of two lineages can be speculated by comparing the amount of sequence variation within each lineage. The date suggested that lineage Nubian underwent a relatively large population expansion than that of lineage Somali in Chinese donkeys (Figure 5). In addition, we examined the demographic expansion for combined dataset of Chinese donkeys and previously reported sequences, and the result also showed the large expansion events in lineage Nubian like in Chinese donkeys (Figure 5). The mtDNA data of lineages Somali and Nubian in Chinese donkeys had a significantly negative *Fs* value. These *Fs* value were -7.64 ($p<0.01$) and -13.12 ($p<0.01$), respectively. These results closely paralleled to the population expansion drawn from those mismatch distribution (Figure 5).

DISCUSSION

This study presents the first substantial analysis of mtDNA diversity in Chinese donkeys and provides 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, 1987). 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, 1987). However, in our 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 (Figure 3). It is worth noting that 11 of 12 Chinese donkey breeds have two divergent lineages Somali and Nubian, showing the obvious introgression process (Figure 1).

Our phylogenetic analysis revealed almost no geographical structure among Chinese domestic donkey breeds. The lack of obvious geographic and body size structures in Chinese donkeys implicated the high gene flow in the Chinese history of human population migration. Our study is consistent with the results of Beja-Pereira et al. (2004). This phenomenon is found also in goats in China (Chen et al., 2005). Our analysis also revealed abundant genetic diversity within breeds and among breeds. The rich genetic diversity existing in Chinese donkey breeds is favorable to preserve the precious genetic resources. However, the breeds that included both lineages Somali and Nubian had an extremely higher genetic diversity than that of Liangzhou breed, which only contained lineage Somali (Table 2). Interestingly, the endangered breed, such as Guanzhong and Beiyang, did not indicate lower genetic diversity.

To determine the spread routes of Chinese donkeys, we compared the mean number of pairwise differences, haplotype diversity and nucleotide diversity of sequence data from all 126 Chinese donkeys. When an ancestral population and a derived population of the ancestral population are compared, genetic loci from the ancestral population are expected to show higher haplotype, nucleotide diversity and mean number of pairwise differences (Savolainen et al., 2002; Troy et al., 2001; Chen et al., 2005). The haplotype, nucleotide diversity and mean number of pairwise differences of Xinjiang breed are highest in Chinese donkey breeds (Table 2). The haplotype diversity of Yunnan, Qingyang, Xiji, Mongolia and Taihang breeds is moderate. Other breeds have lower genetic diversity. Our results support the following hypothesis for the evolution and spread of Chinese donkeys: the earliest domestic donkeys in China were raised in Xinjiang region, the possible spread route were. (1) the spread of Chinese domestic donkeys in history was from Xinjiang via Ningxia, Gansu to Guanzhong plain of Shaanxi province (the Chang'an, now Xi'an city was the center of politics, economy and culture in ancient China); (2) at the same time, Chinese domestic donkeys were parallel from Xinjiang to Inner Mongolian and Yunnan province; (3) at last Chinese domestic donkeys were from Guanzhong plain to other regions of China. The hypotheis is almost consistent with the history records (Xie, 1987). But it is necessary to point out that the population size for each breed in our study is not enough to make conclusion on spread routes of Chinese donkeys.

In 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. Our results assumed that the earliest domestic donkeys in China were raised in Xinjiang region and the possible spread routes of Chinese domestic donkeys in history were from Xinjiang via Ningxia, Gansu to Guanzhong plain, then to other regions of China. However, further information from more extensive samples, microsatellite loci and Y chromosome was necessary to confirm these results.

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