



## Chinese Holstein Cattle Shows a Genetic Contribution from Native Asian Cattle Breeds: A Study of Shared Haplotypes and Demographic History

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**ABSTRACT** : The Chinese Holstein cattle breed, an introduced breed in China, has been crossbred with native cattle breeds. We hypothesised that the Chinese Holstein local population in Beijing share haplotypes with native Asian cattle breeds, the result of a sudden population expansion in the recent past. We also hypothesised that crossbreeding and population expansion left traces that shaped the genetic makeup of the breed. Evaluation of this was performed by mitochondrial DNA (mtDNA) sequence analysis of Chinese Holstein cattle from Beijing ( $n = 41$ ) and a comparison of them with the published mtDNA sequences ( $n = 293$ ) of 14 Asian breeds with an emphasis on Chinese native cattle breeds. Three shared common haplotypes between Chinese Holstein cattle and native Asian cattle were found. Moreover, a high level of haplotype diversity in Chinese Holstein cattle ( $h = 0.9557$ ) and low nucleotide diversity ( $\pi = 0.0052$ ) was found, indicating a past population bottleneck followed by rapid population growth. These findings are supported by the significantly negative deviation of Tajima's  $D$  ( $-1.82085$ ), the star-like pattern of dominant haplotypes and the pairwise mismatch distribution analysis, which showed a unimodal pattern. (**Key Words** : Chinese Holstein Cattle, Native Asian Cattle, Mitochondrial DNA, Demographic History, Mismatch Distribution)

### INTRODUCTION

China's role in international dairy markets has grown significantly in the last 15 years (Fuller et al., 2006), and domestic production is projected to continue to increase (Dong, 2006). This economic and production growth is the reason that China's dairy cattle population has increased in the recent past. To increase milk production, several new breeds were introduced to expand China's native dairy cattle gene pool of 28 described breeds (Qiu et al., 1988). It was previously reported that the Chinese Holstein cattle breed, an introduced breed from Canada, the USA, France and northern Europe, has been crossbred with Chinese native cattle (Liu et al., 2002).

The contribution of native Asian cattle breeds to the genetic diversity and makeup of Chinese Holstein cattle is

unknown. Because understanding the population genetic structure of Chinese Holstein cattle is essential in developing effective strategies for dairy management, we conducted a demographic history study of the breed.

We used mitochondrial DNA (mtDNA) as a trace marker because of its maternal inheritance; it has a faster evolutionary rate compared to nuclear DNA and lacks intermolecular genetic recombination. Mitochondrial DNA has been widely used for studying population structure, phylogeography and phylogenetic relationships at various taxonomic levels (Avice, 2000). The mtDNA displacement loop (D-loop) has been used extensively to evaluate intraspecies variation of Asian cattle breeds (Mannen et al., 2004; Lai et al., 2006; Jia et al., 2007). As intraspecies relationships, in contrast to interspecies relationships, are not hierarchical (Posada and Crandall, 2001), we used a network instead of a phylogenetic tree. The advantage of a network is that conflicts among different sites can be revealed.

Our general assumptions were that the studied Beijing population differed in mtDNA sequence composition and shared haplotypes with native Asian cattle breeds. Based on

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these assumptions, our aims were the following: i) assess which native breeds contributed to the Chinese Holstein mtDNA diversity, ii) display possible ancestral haplotypes and iii) reveal the demographic history of the introduced breed.

## MATERIAL AND METHODS

### Sample and data collection

Blood samples ( $n = 41$ ) were collected by tail-vein puncture from one dairy farm in Beijing (China) and stored in EDTA. Some sequences used had been previously described by us and deposited in GenBank under the accession numbers HM637893-HM637905. Additional haplotypes, obtained through this study, were deposited under the accession numbers HQ115743-HQ115755. Data mining for additional D-loop sequences in Asian cattle breeds was performed on the NCBI nucleotide database, with emphasis on native Chinese cattle ( $n = 293$ ). A table of all sequences, with their accession numbers, is available upon request.

### Mitochondrial DNA sequence analysis

Extraction of DNA was performed with the TIAamp Blood DNA Kit (Tiangen Biotech, Co., Ltd., Beijing) according to the manufacturer's protocol. The primer pair used for specific amplification (5'-CTGCAGTCTC ACCATCAACC-3' and 5'-GATTATAGAACAGGC TCCTC-3') was generated with Primer Premier v.6.0 (Singh et al., 1998). PCR reactions were carried out in 50- $\mu$ l reaction volumes with 1 min denaturation at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C, 30 s at 72°C and finally extension for 10 min at 72°C. The products were purified and sequenced on an ABI 3730 automated sequencer at Beijing Sunbiotech Inc., Beijing. The obtained chromatographic sequences were verified by eye and aligned using the program ClustalW (Larkin et al., 2007). The program DNAsp v.5 (Librado and Rozas, 2009) was used to analyse the alignment and compute haplotype ( $h$ ) (Nei, 1987) and nucleotide ( $\pi$ ) (Nei and Tajima, 1981) diversity. The median network was computed by TCS 1.21 (Clement et al., 2000) using equations as defined previously (Templeton et al., 1992).

### Distribution analyses

Pairwise mismatch distribution analyses of haplotypes (Rogers and Harpending, 1992), computed under a population growth–decline model in DnaSP v.5.0 (Librado and Rozas, 2009), were used to determine whether the Chinese Holstein cattle breed had undergone a sudden population expansion. In this analysis, multimodal distributions corresponded to demographic stability, while sudden expansion generated a unimodal pattern (Slatkin,

1991; Rogers and Harpending, 1992). The historical demographic pattern of studied taxa was examined using neutrality tests and mismatch distribution analysis (Fu, 1997; Tajima, 1989). Coalescent-based Tajima's  $D$  was calculated to test for selective neutrality (Tajima, 1989).

## RESULTS

### Mitochondrial sequence analysis and haplotype network

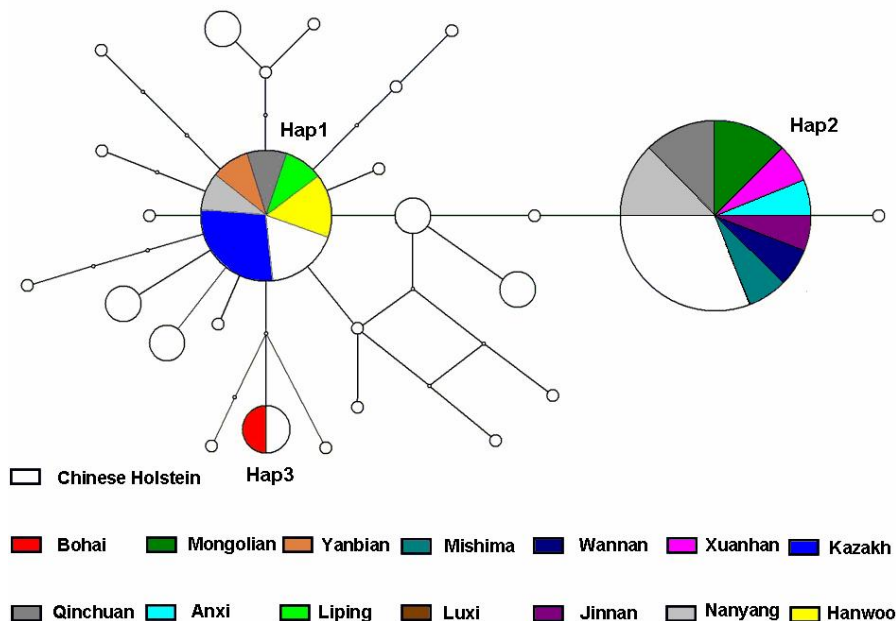
Intrapopulation diversity indices revealed high levels of haplotype diversity in Chinese Holstein cattle ( $h = 0.9557$ ) and low nucleotide diversity ( $\pi = 0.0052$ ). The average number of nucleotide differences ( $k$ ) was 3.695. This indicates a population bottleneck followed by rapid population growth (Avise, 2000).

In the entire dataset ( $n = 334$ ), we identified 159 haplotypes. A minimum spanning network was applied to discover shared haplotypes between the Beijing population and other Chinese native cattle plus Korean and Japanese breeds (Figure 1). The partial data set ( $n = 63$ ) revealed 26 haplotypes found in 41 individuals of Chinese Holstein cattle from Beijing and 22 individuals from other Asian breeds as indicated in Figure 1 as Hap1-3. Of these 22 haplotype-sharing individuals, 19 were from native Chinese breeds, two were from a Korean breed (Hanwoo) and one was from a Japanese breed (Mishima). We found that Hap1-3 were the numerically prominent haplotypes (these accounted for 43.75% of the sequences), and almost all single haplotypes corresponded to one of these main haplotypes by a single or few mutations. Moreover, Hap1 and Hap2 (present in seven and nine breeds, respectively) are the numerical dominating haplotypes. The Table 1 provides the accession numbers of all individuals which share haplotypes with the Chinese Holstein cattle.

### Demographic history

Tajima's  $D$ , which measures the disparity between the number of segregating sites and the pairwise genetic distance, resulted in negative values for both datasets. The entire D-loop dataset ( $n = 334$ ) was not significant (Tajima's  $D$ : -0.56246), meaning that there is no indication for non-neutral evolution. In contrast, Tajima's  $D$  for the Beijing data set ( $n = 63$ ), showed a significant result (-1.82085;  $p < 0.05$ ). Significant negative  $D$  statistics can be interpreted as the signature of a population change in the past, consistent with the population expansion model (Tajima, 1989).

Applied mismatch distribution analysis with the D-loop sequences for the whole dataset displayed a bimodal pattern with two peak signals (Figure 2A). In contrast, the studied population alone with shared haplotypes displayed a unimodal, slightly bell-shaped curve (Figure 2B). The observed bimodal distribution pattern suggests that the samples originated from populations at demographic



**Figure 1.** Median-joining network based on the mitochondrial D-loop region (706 bp). Median-joining haplotype network of n = 63 *Bos taurus* individuals from 14 Asian cattle breeds and the introduced Chinese Holstein cattle breed revealed three shared haplotypes that are numerically dominant. Each circle represents a different haplotype with the size proportional to their relative frequency.

**Table 1.** Accession numbers of all individuals from native Asian cattle breeds which share haplotypes with the Chinese Holstein cattle

Shared haplotypes	Accession number	Breed	
Hap1	AY119673	Qinchuan	
	DQ166083	Qinchuan	
	AB117077	Mongolian	
	AB117076	Mongolian	
	AY521076	Anxi	
	AY115525	Nanyang	
	DQ166101	Nanyang	
	DQ344962	Xuanhan	
	AY521121	Wannan	
	AY521101	Jinnan	
	AB177788	Mishima	
	Hap2	DQ166051	Kazakh
		DQ166049	Kazakh
DQ166052		Kazakh	
DQ166088		Qinchuan	
DQ344948		Luxi	
DQ166061		Yanbian	
AY515626		Liping	
AB117058		Han Woo	
AB117044		Han Woo	
Hap3		DQ344929	Bohai
	DQ166071	Bohai	

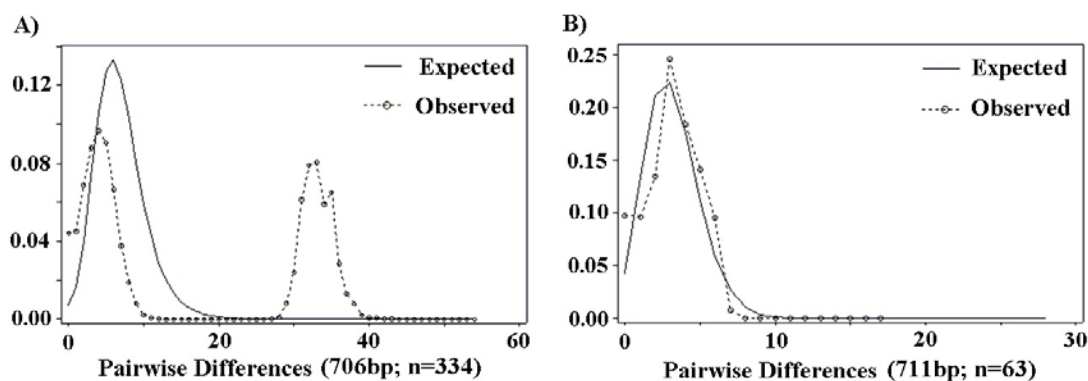
The shared haplotypes are numbered as indicated in Figure 1 from Hap1-Hap3.

equilibrium, whereas the unimodal distribution pattern suggests that the samples originated from a population following a recent demographic and range expansion (Rogers and Harpending, 1992; Ray et al., 2003).

## DISCUSSION

The Chinese Holstein cattle population from Beijing showed a high level of haplotype diversity ( $h = 0.9695$ ) and low nucleotide diversity ( $\pi = 0.0052$ ) for the D-loop region. This genetic variation pattern was largely similar to the haplotype diversity of native Chinese cattle, but exhibited a lower nucleotide diversity in comparison with former studies (Lei et al., 2006; Cai et al., 2010). To explain the maintenance of high haplotypic diversity within populations, several scenarios have been proposed, including: large population size, environmental heterogeneity, and life history traits that favour rapid population expansion (Nei, 1987). Large population size, thought to be responsible for extraordinarily high levels of genetic diversity (Awise et al., 1998) and high haplotypic diversity, suggests a large, stable and effective population size over time (Stepien, 1999). The pattern of genetic variability with high haplotypic diversity, but low nucleotide diversity in the mtDNA D-loop region might imply that the population experienced expansion after the bottleneck (Slatkin and Muirhead, 1999). One explanation could be that in the past 30 years, the economic growth and the change of consumer behaviour led to an increase in the Chinese Holstein cattle population.

The star-like structure of the minimum spanning



**Figure 2.** Mismatch distribution of observed mitochondrial D-loop frequencies compared to the expected frequencies for population sizes. A) The shared haplotype set includes 41 Chinese Holstein cattle from Beijing and 23 haplotypes from native Asian cattle breeds with shared haplotypes showing a unimodal distribution pattern. B) The entire dataset ( $n = 334$ ) includes 293 individuals from 26 native Asian breeds and 41 individuals from Chinese Holstein cattle and shows a multimodal pattern.

network demonstrates that most variants of haplotypes surrounding the central haplotype (Hap1). This pattern suggests that most haplotypes originated recently and is indicative of a population expansion during the recent history of the species (Richards et al., 1998). Furthermore, the numerical dominance of Hap1 and Hap2 (present in seven and nine breeds, respectively) suggests that it is ancestral, or plesiomorphic. This is similar to reports of other mammalian domesticated species (Chen et al., 2006; Liu, 2006). Moreover, this star-like structure can be explained by the introduction of the Chinese Holstein cattle breed to China in the recent past. Population demographic expansion leads to star-like genealogies (Slatkin, 1991), an excess of rare mutations, and unimodal mismatch distributions (Rogers and Harpending, 1992). Population range expansion, however, can lead to the same molecular signature as population demographic expansion if the migration rate between subpopulations is large (Ray et al., 2003). Therefore, both demographic and range expansion might have had an effect on the genetic diversity pattern of Chinese Holstein cattle. In addition, Tajima's  $D$  for the studied population with shared haplotypes ( $n = 63$ ) showed a significantly negative deviation of  $D$  (-1.82085;  $p < 0.05$ ) and indicates a population change in the past (Tajima, 1989).

The mismatch analysis produced a unimodal distribution of pairwise differences for the shared haplotype set. In contrast, the entire dataset showed a multimodal distribution of pairwise differences (Figure 1). In general, an unimodal mismatch distribution indicates a recent range expansion; a multimodal (including bimodal) mismatch distribution indicates diminishing population sizes or structured size; and a ragged distribution suggests that the lineage was widespread (Excoffier et al., 1992; Rogers and Harpending, 1992; Rogers et al., 1996; Excoffier and Schneider, 1999). The unimodal distribution pattern suggests that the samples originated from a population following a recent demographic and range expansion

(Rogers and Harpending, 1992; Ray et al., 2003). The multimodal distribution may also indicate that the population is influenced by migration, is subdivided, and/or has undergone historical contraction (Marjoram and Donnelly, 1994; Bertorelle and Slatkin, 1995; Ray et al., 2003). Graphs of the mismatch distribution demonstrate a significant unimodal distribution indicating expansive population growth for all lineages.

In conclusion, these results indicate that a considerable number of Chinese Holstein cattle are descendants from native Chinese cattle breeds. This is similar to a former study from Japan that showed that Japanese Holstein cows are partly descended from native Japanese cows (Tsuji et al., 2004). Based on the significantly negative deviation of Tajima's  $D$  value, the unimodal mismatch distribution, the high  $h$  and the low  $\pi$ , the current study suggests that the Chinese Holstein cattle breed has experienced a recent rapid population expansion.

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