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Altitude Pressure on the Genetic Variation of Myostatin Gene in *Bos* Species in China

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Abstract: Using the methods of PCR amplification and sequencing, variations of the myostatin (MSTN) gene were detected in 4 Chinese *Bos* sp., normal cattle, zebu, yak and gayal. The results showed that, 375, 372 and 381 base pairs were detected, respectively in the three exons of the MSTN gene in the 4 Bos populations, 8 nucleotide polymorphic sites were observed with 15 haplotypes defined. The nucleotide diversities within the 4 sp. (Pi) ranged from 0.00036-0.00103 and between the 4 species (Dxy) ranged from 0.00076-0.00322. The relation between altitude and the nucleotide diversities indicated that altitude exerted a certain pressure on the genetic variations of the MSTN gene.

Key words: Myostatin, *Bos*, nucleotide polymorphism

INTRODUCTION

Recent years saw many researches relating the divergence in nuclear DNA and mtDNA loci in *Bovinae* livestock in China. However, the causes for these divergences attract little attention. The genes of concern include MSTN (Meng *et al*., 2004) and Cyto-b (Ma *et al*., 2007). As an important nuclear locus, MSTN is also called double-muscling gene, the mutation of some nucleotides in the exons would cause the loss of its function and lead to a double-muscled trait in cattle (McPherron *et al*., 1997a), like in the Belgian Blue and Piedmontese cattle breeds (McPherron and Lee, 1997b; Kambadur *et al*., 1997), in which some polymorphic sites occur at high frequencies. Investigation on the altitude of the habitats of these double-muscled cattle breeds leads to an interesting question, does the altitude of the habitats correlate with the mutation frequencies? Luckily and coincidentally, the mutation rate of MSTN is high among all the nuclear functional genes. The distribution of these mutations across the exon sequences would be a key to explore the relationship between neutral molecular evolution and adaptive evolution, also the possible pressure of altitude. The goal of this study was to collect the information of the mutation in MSTN gene in 4 Chinese *Bos* sp. and to provide some experimental data in elucidating part of the process in the evolution of Chinese *Bos* sp. and molecular evolution of MSTN gene.

MATERIALS AND METHODS

Animal populations and genomic DNA extraction: Using simple random sampling in typical colony methods in the central area of habitat, 18 Mongolia cattle (MG, *Bos taurus*) blood samples were collected from Hejing county Bayibuluke district, Bayingolin Mongolian minority autonomous region, Xinjiang; 18 Leiqiong cattle (LQ, *Bos indicus*) blood samples from Helu village of Nanxing and Changping village of Longmen, Leizhou, Guangdong province; 19 Bayingolin yak (MN, *Bos grunniens*) blood samples were collected from Hejing county of Bayibuluke district, Bayingolin Mongolian minority autonomous region, Xinjiang; and 12 Dulong gayal (DL, *Bos frontalis*) blood samples were collected from Dulong Breed Protection and Reproduction Base in Lushui county of Nujiang State, Yunnan Province. The genome DNA was extracted by the method in literature and stored at -30°C.

Polymerase chain reaction: Exon sequences and part of intron sequences were amplified from genomic DNA. GenBank sequence of *Bos taurus* (GenBank Accession No: AB076403) was used to design primers (Tay, 2004) (Shanghai Genecore company). The primers were listed below. In a typical reaction, 25 μL PCR system contained 10×PCR buffer 2.5 μL, Mg^{2+} 2.5 μM, dNTP 400 μM, Taq DNA enzyme 1U (Takara company, China), Forward and Reverse primer 0.4 μM each, template DNA 1 μL. The amplification program consisted of predenaturation (94°C, 3 min), followed by 34 cycles of denaturation (94°C, 0.5 min), primer annealing (51°C, 0.5 min) and extension (72°C, 0.5 min) and a final extension (72°C, 3 min). The PCR products were stored at 4°C.

P1	Forward	5'- GGCTTGGCGT TACTCAAAAGC-3'
	Reverse	5'- CTCCTCCTTACGTACAAGCCAGCA-3'
P2	Forward	5'-GTTCATAGATTGATATGGAGGTGTTCG-3'
	Reverse	5'-ATAAGCACAGGAAACTGGTAGTTATT-3'
P3	Forward	5'-GAAATGTGACATAAGCAAAATGATTAG-3'
	Reverse	5'-ATACTCTAGGCTTATAGCCTGTGGT-3'

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Sequencing and alignment of PCR fragments: Three exons are separated by two introns in the bovine MSTN gene. PCR products were first detected using 1.2% agarose gel or 10% PAGE gel and then sequenced (SANGON Company, Shanghai, China). The sequencing results were analyzed through DNAstar, MEGA 3.1 and DnaSP software to describe the nucleotide divergences of MSTN gene between and among *Bos* populations.

Regression analysis between altitude and nucleotide diversity: The data of the altitude for the habitats of the 4 Chinese Bos species were collected from literature and was calculated mean of the lower and upper limit for each population. The correlation between altitude and nucleotide diversity was analyzed by regression analysis using SPSS software.

RESULTS

PCR amplification and sequencing: The designed primers were used to amplify all the DNA samples. The size of each PCR band was similar to literature (Tay, 2004). The sequences of PCR products for the 66 DNA samples contained exon 1 (375 bp), exon 2 (372 bp) and exon 3 (381 bp), which are identified with the reported exons in literature (Tay, 2004).

Sequence analysis: The average content for each base of MSTN exons was similar in each population (Table 1).

No significant biases of base content were detected in any population. Eight nucleotide sites were observed polymorphic in the 4 *Bos* populations with totally 15 haplotypes. One nucleotide site was observed polymorphic in Mongolia population, three sites in the Leiqiong population, six in the Bazhou yak population and three in the Dulong population (Table 2). Mongolia population in the study held 2 haplotypes, Leiqiong population 7 haplotypes, Bazhou yak population 6 haplotypes and Dulong population 2 haplotypes. Among these haplotypes, Mongolia and Leiqiong population shared one common haplotype, occupying 30.6% of the both populations. Bazhou yak and Dulong population shared another common haplotype, occupying 13.3% of the both populations. Moreover, all the haplotypes of the Bazhou yak population had two special bases (nt 612, nt 639) other than Mongolia and Leiqiong populations.

The detected nucleotide variations included six transitions and two transversions, all of which were at the third codon but one (A704G) in the Bazhou yak population at the second codon with a change of amino acid His to Arg (H 235 R).

Table 1: Nucleotide composition of MSTN exons in 4 Chinese *Bos* sp. (%)

Population	T	C	A	G	Total (bp)
MG	25.4	21.3	31.2	22.2	1128
LQ	25.4	21.3	31.1	22.2	1128
MN	25.3	21.4	31.4	22.0	1128
DL	25.4	21.3	31.4	22.0	1128

Table 2: Nucleotide variance of MSTN exons in 4 Chinese *Bos* species (1128 bp)

Population	1 1 1	4 1 4	6 1 2	6 3 9	7 0 4	7 9 8	1 0 7 7	1 0 8 3	Ratio with population (%)
MG 1	G	C	G	G	A	A	A	C	55.60
MG 4	•	T	•	•	•	•	•	•	44.40
LQ 14	•	•	•	•	•	•	•	•	5.55
LQ 9	•	•	•	•	•	•	C	•	11.10
LQ 2	C	•	•	•	•	•	C	•	5.55
LQ 1	•	•	•	•	•	•	C	T	25.00
LQ 3	•	•	•	•	•	•	•	T	8.35
LQ 5	C	•	•	•	•	•	•	T	8.35
LQ 4	C	•	•	•	•	•	C	T	36.10
MN 1	•	•	A	A	G	•	•	•	11.10
MN 2	•	•	A	A	•	•	•	•	66.70
MN 4	•	•	A	A	G	G	•	T	5.55
MN 6	•	•	A	A	•	•	•	T	5.55
MN 09	•	T	A	A	•	G	•	T	5.55
MN 10	•	•	A	A	G	G	•	•	5.55
DL 1	•	•	A	A	•	•	•	T	25.00
DL 3	•	•	A	•	•	•	•	•	75.00

Table 3: Inner population nucleotide variation of MSTN exons in 4 Chinese *Bos* sp.

Species	n	Size (bp)	S	Pi	Hap	NHap
Bos taurus	18	1128	1	0.00046	0.523	2
Bos indicus	18	1128	3	0.00103	0.614	7
Bos frontalis	12	1128	1	0.00036	0.409	2
Bos grunniens	18	1128	4	0.00094	0.562	6

DNA divergence within and between populations: The sequences of the 66 samples were analyzed by DnaSP software. The inner population nucleotide diversity (Pi) of the Dulong population was the lowest (0.00036) and Leiqiong population the highest (0.00103) (Table 3); the nucleotide diversity between Bazhou yak population and Dulong population (Dxy) was the lowest (0.00076) and the nucleotide diversity between Bazhou yak population and Leiqiong population (Dxy) the highest (0.00322) (Table 4).

Regression analysis: The original central habitat of Leiqiong population was located in low hilly regions of Leizhou Peninsula, Guangdong province and coastal regions of Hainan Island. The altitude is about 100 m high, which is a good base point and therefore Leiqiong population is a reference population. The data of altitude and the nucleotide diversities between Leiqiong population and the other populations (Dxy) were compared (Table 5). The Dxy values correlated

Table 4: The nucleotide diversity of MSTN exons between 4 Chinese Bos populations (Dxy)

Species	*Bos taurus*	*Bos indicus*	*Bos frontalis*	*Bos grunniens*
Bos taurus				
Bos indicus	0.00241			
Bos frontalis	0.00150	0.00313		
Bos grunniens	0.00178	0.00322	0.00076	

Table 5: Altitude and Dxy between Leiqiong and the other populations

Species	Altitude mean (range) (m)	Dxy
Bos indicus	100	0
Bos taurus	1250 (1000-1500)	0.00241
Bos frontalis	1950 (600 or 1200-3000)	0.00313
Bos grunniens	4000 (3000-5000)	0.00322

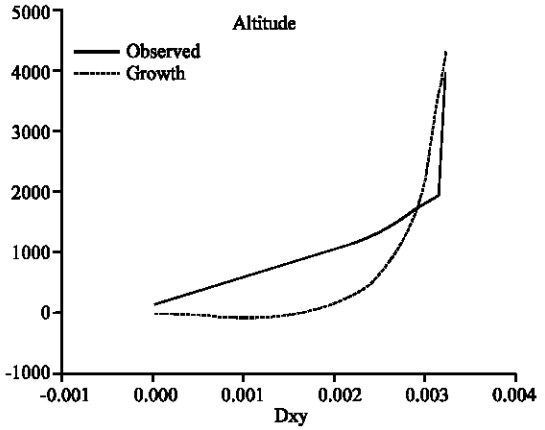

Fig. 1: The observed curve and regression curve by growth model

significantly ($p<0.05$) with the altitude of the central habitats for each population. Regression curve showed a similarity between the observed curve and logarithmic regression curve (Fig. 1).

DISCUSSION

The origin of Dulong gayal (*Bos frontalis*) aroused some concern and dispute. Winter H.B proposed it could be the offspring of male gaur and female zebu (Winter *et al*., 1984), while the local folks recorded that the Dulong gayal was a semi-domesticated type from capture and long-term domestication of wild gaur. According to the analysis of the gayal MSTN exons in the study, gayal population had a low level of genetic divergence like reports in China (Nie *et al*., 1995), though the absolute value of Pi was different from the value based on the Cyt b gene. The low divergence was because they inhabit in the mountainous regions of Yunnan and Tibet, with the characteristics of high altitude and geographical separation. Because the population size of Dulong cattle was rather small three decades ago with a population of about 77, the genetic drift and environmental disturbance had long been affecting the genetic situation. Some F_1 hybrids of gayal and local yellow cattle were reported with a good growth performance (Kai-dian *et al*., 2003). The findings in our study together with the related reports demonstrated that the Dulong gayal resource was under certain destruction, requiring effective protection and avoiding hybridization.

All the MSTN exons in Bos populations take up only a small part of the whole MSTN gene. On the analysis of coding regions in the study, the Bazhou yak population had two special bases (nt 612, nt 639) other than Mongolia and Leiqiong populations, demonstrating that the divergence between the Bazhou yak and Mongolia or Leiqiong population was significant, which provided a certain support for regarding yak population as an individual genus. Generally, the geographical distribution of species has reasons. Interspecies competition is a key reason; environmental factors like temperature and precipitation are also the causes for the distribution. With a comprehensive force of several environmental factors, altitude exerts its force on selection of adaptivity. Anti-coldness and muscularity are two important traits supporting survival and adaptivity, especially in the high-altitude mountainous regions.

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