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GENETIC VARIATION OF SRY GENE IN YAK AND RELATED BOVINES

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ABSTRACT

Y-chromosome markers have high value for the reconstruction of the male's genetic and phylogeographic history. Genetic polymorphism and diversity of SRY gene in yak *(Bos grunniens)*, yak hybrids and hill cattle of Arunachal Pradesh was investigated in the present study. Polymerase chain reaction and direct DNA sequencing was performed to find out the nucleotide differences between the species. The results of the multiple sequence alignment revealed that there exists variation at different positions viz., 61, 122 and 197.

KEY WORDS

Y Chromosomal genes, Single Nucleotide Polymorphisms

INTRODUCTION

Yak (*Peophagus grunniens*) is a unique bovine species living in the difficult terrains, which appeared some two million years ago[1] (Rongchang *et al.,* 1994). In India, 65000 yaks and their hybrids are found in the hilly tracts of Arunachal Pradesh, Sikkim, Jammu and Kashmir and Himachal Pradesh. Y-chromosomal loci exhibit an accelerated evolutionary rate compared to orthologous sequences on the X chromosome[2, 3] (Shimmin *et al*., 1993, Huang *et al.,* 1997) and have markers of high potential value for the reconstruction of the male's genetic and phylogeographic history. SRY gene is an intronless gene[4] (Su and Lau, 1993) and required to initiate testis development[5] (Koopman *et al*., 1991) and is present in Y chromosome. Polymorphisms in Y-chromosomal genes are scarce and sequence variants of the SRY gene are stable[6] (Verkaar *et al*., 2003). Utilizing A→G diagnostic mutation at position 936 of SRY gene (Genbank AB039748). Ramesha *et al.* (2009) developed a simple *Ssp*1-PCR-RFLP assay to identify the paternal species in yak and cattle hybrids of different filial generations through species specific paternally transmitted Y-chromosomal markers[7]. The *Ssp1*-PCR-RFLP assay of SRY gene could be used to identify male mediated introgression. The search of Gen bank for SRY gene sequence in yaks indicated only 8 accession numbers viz., FJ373272, DQ336531, EF693876, EF693886, EU547257, AY079144, AB077320 and AF148463. No information is available about the Indian yaks. Yak herders in India practice species hybridization to utilize hybrid vigour[8] (Ramesha *et al*., 2008). Yak females are mated by hill cattle bulls and vice versa to produce hybrids. The male hybrids including backcross males are sterile but female hybrids are fertile. F_1 hybrid females (Dzomo) are backcrossed to bulls from either of the parental species. Considering the role of the SRY gene in male fertility, this study was undertaken to find out the genetic polymorphism of SRY gene in yaks, yak hybrids and hill cattle of Arunachal Pradesh.

MATERIALS AND METHODS

Random blood samples (10 ml) were collected from yak males (N=20), yak hybrids (15 yak x cattle) and hill cattle (N=18) of Arunachal Pradesh which were considered as representative of the existing gene pool of the population. Genomic DNA was isolated from whole blood by High Salt method[9] (Montgomery and Sise, 1990). After checking the quality and quantity of DNA, it was diluted to a final concentration of 50ng/µl and stored at 4°C. The polymerase chain reaction (PCR) was carried out using the primers (F-5'GTCTGCTGCACCTTCATCCT3' and R-5'GTTCATGGGTCGCTTGACGT3')[3] reported by Verkaar *et al.* (2003). The total reaction mix of 50 µl contained 2 µl of template DNA, 1µl (100 pmol) each of forward and reverse primer, 2 µl (150 µM) each of dNTP, 2 unit of *Taq* DNA polymerase (Sigma, USA) and 1X PCR buffer and distilled water. Amplifications were carried out in a thermal cycler (ABS, USA) for 30 cycles with 94^{o}C for 30s, 58.5^{o}C for 45s and 72^{o}C for 45s with initial denaturation at 95^{o}C for 5 minutes and final extension at 72^{o}C for 8 minutes. The amplified products of the SRY gene were detected on 2% agarose gel using loading dye, electrophoresed and visualized using UV light after ethidium bromide staining. The PCR products were resolved by SSCP analysis[10] as described by Markoff *et al*. (1997) with minor modifications using vertical slab gel unit (Biometra, USA). Various factors such as the amount of PCR product, denaturing solution and time, acrylamide concentration, glycerol, voltage, running time and temperature were optimized for SSCP analysis. Each PCR product was diluted in a denaturing solution

(95% formamide, 10 mM NAOH, 0.05% bromophenol blue, 20mM EDTA) denatured at 95^0C for10 minutes. After denaturation the samples were quickly chilled on ice and resolved on 9% polyacrylamide gel. The gels were silver stained[11] using the improved procedure as described by Benbouza, *et al.* (2006) with minor modifications. The gels were stained in 0.2 per cent (w/v) silver nitrate solution for 20 min and analyzed for SSCP patterns using Gel documentation system (Syngene, USA). Direct DNA sequencing of two PCR products from each of SSCP pattern belonging to hill cattle (2 pattern: hill cattle 1 & hill cattle 2), hybrids (2 pattern: Dzo 1 pattern, Dzo 2 pattern) and yak (single pattern) were carried out by using the Automated DNA Sequencer to get the complete picture of polymorphism at nucleotide level. Multiple sequence alignment analysis was carried out online (http://xylian.igh.cnrs.fr/msa/msa.html) for alignment of the DNA sequences.

RESULTS AND DISCUSSION

The quality and quantity of DNA was checked by agarose gel electrophoresis and has been found to be of good quality. The observed ratio of OD260 to OD280 was 1.7 to 1.9. The PCR amplification in all DNA samples from cattle, yak and their hybrids generated a 524 bp product of SRY gene. Two SSCP band patterns were observed in hill cattle (hill cattle 1 & hill cattle 2 patterns), hybrids also showed 2 SSCP patterns (Dzo 1 pattern, Dzo 2 pattern) while the entire yaks showed single pattern which was similar to Hill cattle 2 pattern. Only 2 hill cattle showed SSCP pattern similar to SSCP pattern shown in yaks. The multiple sequence alignment (Figure 1) of the DNA sequences of the SRY gene revealed that there exist no variation among the Indian yaks and the template. But, variation has been observed between the Indian yaks, yak hybrids, and hill cattle population at various positions viz., 61, 122 and 197 (Figure 2, Table 1).

Figure 1

CLUSTAL W (1.8) multiple sequence alignment (BioEdit-generated mock-up) of SRY gene in hill cattle, yak and their hybrids. Variations are indicated in red color

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AY079144        CTTTTTGTATTTTTAGCAGGTGATCCTGTTAAGTAGCTTTGCTTGAGAAA
Yak             CTTTTTGTATTTTTAGCAGGTGATCCTGTTAAGTAGCTTTGCTTGAGAAA
Dzo1            CTTTTTGTATTTTTAGCAGGTGATCCTGTTAAGTAGCTTTGCTTGAGAAA
Dzo2            CTTTTTGTATTTTTAGCAGGTGATCCTGTTAAGTAGCTTTGCTTGAGAAA
Hill cattle1    CTTTTTGTATTTTTAGCAGGTGATCCTGTTAAGTAGCTTTGCTTGAGAAA
Hill cattle2    CTTTTTGTATTTTTAGCAGGTGATCCTGTTAAGTAGCTTTGCTTGAGAAA

AY079144        GAGTAGGTTGGTGGGTTTGGGCTGACTGCCAGGACGTATTGAGGGGAGGT
Yak             GAGTAGGTTGGTGGGTTTGGGCTGACTGCCAGGACGTATTGAGGGGAGGT
Dzo1            GAGTAGGTTGGTGGGTTTGGGCTGACTGCCAGGACGTATTGAGGGGAGGT
Dzo2            GAGTAGGTTGATGGGTTTGGGCTGACTGCCAGGACGTATTGAGGGGAGGT
Hill cattle1    GAGTAGGTTGGTGGGTTTGGGCTGACTGCCAGGACGTATTGAGGGGAGGT
Hill cattle2    GAGTAGGTTGATGGGTTTGGGCTGACTGCCAGGACGTATTGAGGGGAGGT

AY079144        ATTGGGGGCGGAGAAATAAATGTTTCACTGTATATATTGCACTAAGTCAG
Yak             ATTGGGGGCGGAGAAATAAATGTTTCACTGTATATATTGCACTAAGTCAG
Dzo1            ATTGGGGGCGGAGAAATAAATGTTTCACTGTATATATTGCACTAAGTCAG
Dzo2            ATTGGGGGCGGAGAAATAAATATTTCACTGTATATATTGCACTAAGTCAG
Hill cattle1    ATTGGGGGCGGAGAAATAAATGTTTCACTGTATATATTGCACTAAGTCAG
Hill cattle2    ATTGGGGGCGGAGAAATAAATATTTCACTGTATATATTGCACTAAGTCAG

AY079144        TCTGTGGTAAGAACAACTTATGAATAGCACCATAATTTTTAGAACGTTTA
Yak             TCTGTGGTAAGAACAACTTATGAATAGCACCATAATTTTTAGAACGTTTA
Dzo1            TCTGTGGTAAGAACAACTTATGAATAGCACCATAATTTTTAGAACGTTTA
Dzo2            TCTGTGGTAAGAACAACTTATGAATAGCACCATAATTTTTAGAACGCTTA
Hill cattle1    TCTGTGGTAAGAACAACTTATGAATAGCACCATAATTTTTAGAACGTTTA
Hill cattle2    TCTGTGGTAAGAACAACTTATGAATAGCACCATAATTTTTAGAACGCTTA
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AY079144        CACCGCATATTACTTCCTCCCCTTTTAAACAGTGCAGTCGTATGCTTCTG
Yak             CACCGCATATTACTTCCTCCCCTTTTAAACAGTGCAGTCGTATGCTTCTG
Dzo1            CACCGCATATTACTTCCTCCCCTTTTAAACAGTGCAGTCGTATGCTTCTG
Dzo2            CACCGCATATTACTTCCTCCCCTTTTAAACAGTGCAGTCGTATGCTTCTG
Hill cattle1    CACCGCATATTACTTCCTCCCCTTTTAAACAGTGCAGTCGTATGCTTCTG
Hill cattle2    CACCGCATATTACTTCCTCCCCTTTTAAACAGTGCAGTCGTATGCTTCTG

AY079144        CTATGTTCAGAGTATTGAACGACGATGTTTACAGTCCAGCTGTGGTACAG
Yak             CTATGTTCAGAGTATTGAACGACGATGTTTACAGTCCAGCTGTGGTACAG
Dzo1            CTATGTTCAGAGTATTGAACGACGATGTTTACAGTCCAGCTGTGGTACAG
Dzo2            CTATGTTCAGAGTATTGAACGACGATGTTTACAGTCCAGCTGTGGTACAG
Hill cattle1    CTATGTTCAGAGTATTGAACGACGATGTTTACAGTCCAGCTGTGGTACAG
Hill cattle2    CTATGTTCAGAGTATTGAACGACGATGTTTACAGTCCAGCTGTGGTACAG

AY079144        CAACAAACTACTCTCGCTTTTAGGAAAGACTCTTCCTTGTGCACAGACAG
Yak             CAACAAACTACTCTCGCTTTTAGGAAAGACTCTTCCTTGTGCACAGACAG
Dzo1            CAACAAACTACTCTCGCTTTTAGGAAAGACTCTTCCTTGTGCACAGACAG
Dzo2            CAACAAACTACTCTCGCTTTTAGGAAAGACTCTTCCTTGTGCACAGACAG
Hill cattle1    CAACAAACTACTCTCGCTTTTAGGAAAGACTCTTCCTTGTGCACAGACAG
Hill cattle2    CAACAAACTACTCTCGCTTTTAGGAAAGACTCTTCCTTGTGCACAGACAG

AY079144        TCATAGCGCAAATGATCAGTGTGAAAGGGGAGAACATGTTAGGGAGAGCA
Yak             TCATAGCGCAAATGATCAGTGTGAAAGGGGAGAACATGTTAGGGAGAGCA
Dzo1            TCATAGCGCAAATGATCAGTGTGAAAGGGGAGAACATGTTAGGGAGAGCA
Dzo2            TCATAGCGCAAATGATCAGTGTGAAAGGGGAGAACATGTTAGGGAGAGCA
Hill cattle1    TCATAGCGCAAATGATCAGTGTGAAAGGGGAGAACATGTTAGGGAGAGCA
Hill cattle2    TCATAGCGCAAATGATCAGTGTGAAAGGGGAGAACATGTTAGGGAGAGCA

AY079144        GCCAGGACCACGTCAGCGACCC
Yak             GCCAGGACCACGTCAGCGACCC
Dzo1            GCCAGGACCACGTCAGCGACCC
Dzo2            GCCAGGACCACGTCAGCGACCC
Hill cattle1    GCCAGGACCACGTCAGCGACCC
Hill cattle2    GCCAGGACCACGTCAGCGACCC
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Figure 2
Chromatogram showing variations among Indian yak, yak hybrids and hill cattle population at various positions

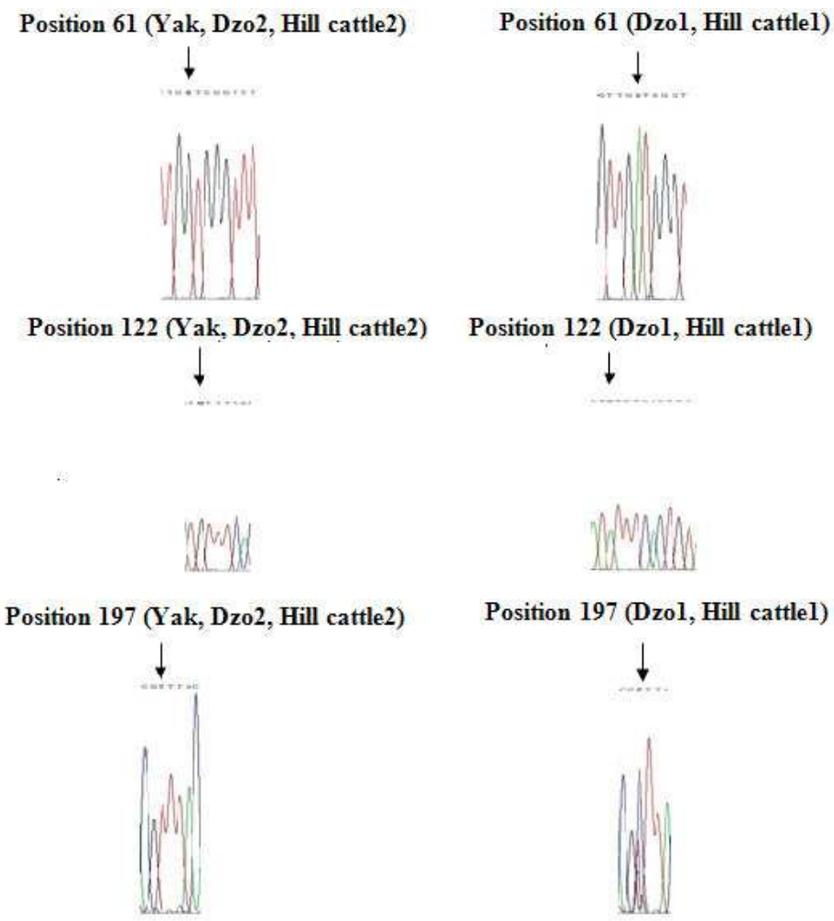

Table 1
Variation in SRY gene sequence in yak, cattle and their hybrids

Sl No.	Position	Dzo 1	Dzo2	Hill cattle1	Hill cattle2	Yak	AY079144
1	61	A	G	A	G	G	G
2	122	A	G	A	G	G	G
3	197	C	T	C	T	T	T

Yak females are mated by cattle bulls and vice versa to produce hybrids. The male hybrids including backcross males are sterile whereas female hybrids are fertile. F_1 hybrid females (Dzomo) are backcrossed to bulls from either of the parental species. Y-chromosomal markers are likely to reveal male introgression through mating of females by males from neighboring habitats. Males are likely to have broader geographic area[12] (Nijman *et al.*, 2003) thus Y-chromosomal markers have broader geographical distribution than the maternal mitochondrial markers. Y-chromosomal markers are useful for detection of species hybridization[14] (Verkaar *et al.*, 2003). Variations in the SRY gene can further be used for the association studies and marker assisted selection. Generally few males often have the

priority to mate with a majority of females in yaks. As a result, the effective population size for males will be smaller than that of females. This unequal effective population size between the sexes would reduce the variation of the Y-chromosome, making the genes on the Y chromosome less divergent. The observed lower divergence of SRY gene may be due to the rapid cladogenesis in the Bovidae family offering little time to accumulate mutation. The evolution of neutral mutations on the Y chromosomal genes is comparatively faster than the genes in the autosomal loci which could be due to the higher number of divisions occurring during spermatogenesis than oogenesis. However, due to gene hitch-hiking (selective sweeps) or background selection, Y chromosomal genes show reduced variability. Polymorphisms in Y-chromosomal genes are scarce and sequence variants of the SRY gene are stable[15] (Verkaar *et al.,* 2003). The DNA sequencing results of the SRY gene revealed that there exist no variation among Indian yaks and the template studied (AY079144) and variation has been observed between the Indian yak, yak hybrids and hill cattle population at various positions.

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REFERENCES

1. Rongchang Z, Jianping W and Jianlin H, Yak production in China, Asian Livestock 19:115-118, (1994).
2. Shimmin LC, Chang BHJ, Hewett-Emmett D and Li WH, Potential problems in estimating the male-to female mutation rate ratio from DNA sequence data, Journal of Molecular Evolution 37:160–166, (1993).
3. Huang W, Chang BHJ, Gu X, Hewett-Emmett D and Li W, Sex differences in mutation rate in higher primates estimated from AMG intron sequences, Journal of Molecular Evolution 44:463–465, (1997).
4. Su H and Lau YFC, Identification of the transcription unit, structural organization, and promoter sequence of the human sex-determining region Y (SRY) gene, using a reverse genetic approach, American Journal of Human Genetics 52: 24-38, (1993).
5. Koopman P, Gubbay J, Vivian N, Good-fellow P and Lovell-Badge R, Male development of chromosomally female mice transgenic for the SRY, Nature 351: 117-121, (1991).
6. Verkaar ELC, Vervaecke H, Roden C, Mendoza LR, Barwegen MW, Susilawati T, Nijman IJ and Lenstra JA Paternally inherited markers in bovine hybrid populations, Heredity 91: 565-569, (2003).
7. Ramesha KP, Das S, Kataktalware MA, Saravanan BC, Pourouchottamane R, Bandopadyaya S, Krishnan G and Sarkar M, Paternally inherited species specific SSP1-PCR-RFLP assay for hybridization analysis in yak-cattle hybrid populations, Indian Veterinary Journal 86:635-637, (2009).
8. Ramesha KP, Kataktalware MA, Das S, Pourouchottamane R, Bandopadyaya S, Saravanan BC, Krishnan G, Sarkar M and Bhattacharya M, Performance and body measurements of yaks and their hybrids under field condition, Indian Journal of Animal Sciences 78(9):1032-1033, (2008).
9. Montgomery GW and Sise JA, Extraction of DNA from sheep white blood cells, New Zealand Journal of Agricultural Research 33: 437-441, (1990).
10. Markoff A, Savov A, Vladimirov V, Bogdanova N, Kremensky I and Ganev V, Clinical Chemistry 43(1):3033, (1997).
11. Benbouza H, Jacquemin J, Baudoin J and Mergeai G, Optimization of a reliable, fast, cheap and sensitive silver staining method

to detect SSR markers in polyacrylamide gels, Biotechnology, Agronomy, Society and Environment 10 (2):77-81, (2006).

12. Nijman IJ, Otsen M, Verkaar ELC, De-Ruijter C, Hanekamp E and Ochieng JW, Hybridization of banteng (Bosjavanicus) and zebu (Bos indicus) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites, Heredity 90: 10–16, (2003).