动 物 学 研 究 2006, Dec. 27(6): 615 – 620 CN 53 – 1040/Q ISSN 0254 – 5853
Zoological Research

# Mitochondrial DNA D-loop Variation and Genetic Background of Brahman Cattle

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**Abstract:** The complete mitochondrial DNA D-loop sequences from 10 stud Brahman cattle were sequenced and analyzed. The results showed that the genetic diversity of Brahman cattle was rich; the rate of nucleotide variation, haplotype diversity and nucleotide diversity were 6.25%, 0.978 ± 0.054 and 0.014 30 ± 0.008 68, respectively. Nine haplotypes were defined and fell into two distinct lineages, suggesting that Brahman cattle have both *Bos indicus* (Zebu) and *B. taurus* genetic background. The taurine haplotypes were predominant at 90% and only Brah-6 belonged to the Asian zebu mt-haplotype. This indicates that Brahman cattle was one of the zebu breeds and inherited the excellent characteristics of both the Asian zebu and European beef cattle, such as easy calf delivery, high quality beef, heat tolerance and resistance to various parasites. Breeders introduced Brahman cattle to improve the productivity and adaptability of native cattle. The Zebu has evidently frequently introgressed into the modern taurine breeds. As for modern zebu breeds, *B. taurus* also highly contributed to their formation, except for the Asian zebu. Furthermore our results also confirm the hypothesis that *B. indicus* has undergone a separate domestication event and originated from the Indian subcontinent.

**Key words:** Brahman cattle; mtDNA D-loop polymorphism; genetic background

# 婆罗门牛 mtDNA D-loop 变异及其遗传背景

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**摘要:** 对 10 头原种婆罗门牛 mtDNA D-loop 全序列 912 bp 测序, 婆罗门牛遗传多样性丰富, 检测到的 9 种单倍型兼有瘤牛(*B. indicus*)与普通牛(*B. taurus*)的遗传背景, 核苷酸变异率为 6.25%, 单倍型多态度为 0.978 ± 0.054, 核苷酸多态度为 0.014 30 ± 0.008 68。所有单倍型聚为明显的两大分支, 婆罗门牛的大部分单倍型为普通牛单倍型类群, 并占绝对优势(90%), 仅 Brah-6 与亚洲瘤牛聚在一起, 属于亚洲瘤牛线粒体单倍型, 表明婆罗门牛的确是集亚洲瘤牛、欧洲普通牛等优良特性于一身(易产犊、产肉性能好、耐热与体表寄生虫等)的瘤牛品种之一。育种学家引种瘤牛的目的是改善当地牛的生产力与适应性, 现代普通牛表现出明显又普遍的瘤牛渐渗现象。对现代的瘤牛品种而言, 除亚洲瘤牛品种外, 普通牛对其他瘤牛品种育成的贡献同样高。支持瘤牛(*B. indicus*)为独立驯化、起源于印度次大陆的假说。

**关键词:** 婆罗门牛;mtDNA D-loop 多态性;遗传背景
**中图分类号:** Q959.842; Q953 **文献标识码:** A **文章编号:** 0254 – 5853(2006)06 – 0615 – 06

* Receivd date: 2006 – 05 – 18; Accepted date: 2006 – 08 – 14
收稿日期: 2006 – 05 – 18; 接受日期: 2006 – 08 – 14
基金项目: 云南省自然科学基金(2003C0011Z, 2004YX41); 云南省科技厅科技项目(2004NG04)
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Domesticated cattle have played a key role in agriculture, economy, culture and religion during the historic and modern societies of human civilization. Zoologists usually classified cattle as two separate species: *B. indicus*, indicine, humped; and *B. taurus*, taurine, humpless. However, the complete inter-fertility between *B. indicus* and *B. taurus* has given rise to their consideration as subspecies (Grigson, 1980; Epstein & Mason, 1984; Payne, 1991), and that all cattle breeds derived from the now extinct wild ox or aurochs, *B. primigenius*. It is well known that the first domestication of cattle was some 8 000 – 10 000 years ago in the Near East. Cattle subsequently dispersed around the world and gave rise to all the modern cattle breeds, excluding Bali and Mithan cattle (Grigson, 1980; Epstein & Mason, 1984; Payne, 1991; Loftus et al, 1999). Loftus et al (1994a, b) hypothesized that Asian zebu had undergone an independent domestication. mtDNA has an evolutionary rate 5 – 10 times faster than typical nucleus genes. In addition, the mitochondrial DNA control region (mtDNA D-loop), has an evolutionary rate which is faster than other mitochondrial regions. MtDNA was used to clarify the origin, domestication and genetic background of domesticated cattle.

Brahman cattle originated from India about 4 000 years ago. As one of the zebu breeds, it is characterized by long floppy ears and a large hump over the top of the shoulder and neck. It has highly developed sweat glands and a dewlap and has good heat tolerance and parasite resistance. They can travel long distances to water and therefore have a larger grazing range. Brahman cattle seldom contract eye cancer and pinkeye. They have a considerable reputation for rapid growth and a high dressing percentage, or the percentage of animals killed for meat (Oklahoma State University Board of Regents, 2000). Based on the above, Brahman cattle were introduced to improve the adaptability and production of local cattle breeds in the mid-18th century. Later, breeders crossbred some beef cattle breeds, including the Beefmaster (1/2 Brahman × 1/4 Shorthorn × 1/4 Hereford), Santa Gertrudis (3/8 Brahman × 5/8 Shorthorn), Braford (3/8 Brahman × 5/8 Hereford). In Yunnan province, China, Brahman cattle were also introduced to cross with Yunnan native yellow cattle in the early 1980s. The aim was to crossbreed a new beef cattle breed (or strains) that could adapt well to the heat and humid weather in South China (Wen et al, 2003). The resulting breed (1/2 Brahman × 1/4 Murray Gray × 1/4 Yunnan Yellow cattle, BMY) is breeding *inter se*. To find out the genetic diversity and background of Brahman cattle, we sequenced the complete mtDNA D-loop. This will cast light on more genetic information about the conservation of breeds in the tropical and subtropical areas of South China.

## 1 Materials and Methods

### 1.1 Materials

The blood from ten Brahman cattle was collected from the tail artery at Xiaoshao Farm of the Yunnan Beef Cattle and Pasture Research Center.

### 1.2 DNA extraction and sequencing

Total DNA was isolated from fresh blood samples as described previously (Yuan et al, 2001). D-loops were amplified on a Perlin-Elmer DNA thermocycler. Primers were designed from the published bovine complete mtDNA sequence (V00654, Anderson et al, 1982). The forward primer was L15718: 5′-CTAAGACTCAAGGAAGAAACTGC-3′ and the reverse primer was H415: 5′-GACTCATCTAGGCATTTTCA-3′. The PCRs were carried out using a standard PCR program with 5 min denaturizing at 95 ℃, 35 cycles for 1 min at 94 ℃, 1 min annealing at 58 ℃, 1 min extension at 72 ℃, and final extension for 10 min at 72 ℃. To ensure the accuracy of the sequences, we also used another two primers when sequencing reactions were done, H16320: 5′-TTAAGAGGAAAGAATGGAC-3′ and L16230: 5′-ACCGTGGGGGTCGCTAT-3′. Purification of PCR products and sequencing reactions followed Qu et al (2003).

### 1.3 Data analysis

Data analysis was processed according to Qu et al (2003). The NJ tree was constructed using the software Mega 2.1 (Kumar et al, 1993).

## 2 Results and Analyses

### 2.1 Variation in the mtDNA D-loop of Brahman cattle

Nine hundred and twelve bp complete mitochondrial DNA D-loops of ten Brahman cattle was sequenced and 57 polymorphic sites were found (GenBank accession numbers DQ887760 – DQ887768). There was a variation in length of a 6 – 8 bp poly (C) tract found at 764 bp site. The variance rate of nucleotides was 6.25%. Three of the 57 substitutions were due to transversions, reflecting a heavy transitional bias, which was congruent with previous bovine con-

trol region variations (Loftus et al, 1994a; Bradley et al, 1996; Mannen et al, 1998). The three transversions were at 150 bp, 162 bp and 831 bp. There were also four 1 – bp insertion/deletions at 352 bp, 410 bp, 770 bp and 771 bp, respectively (Fig. 1). Brahman cattle showed high genetic diversity, of which haplotype diversity, nucleotide diversity and nucleotide difference were $0.978 \pm 0.054$, $0.014\,30 \pm 0.00\,868$ and $13.000 \pm 8.232$, respectively. The Tajima's $D$ value was $-1.4982$ ($P > 0.10$), which was in accordance with the neutral mutation hypothesis. By aligning the sequences nine haplotypes were defined, of which Brah-6 belonged to the classical Asian zebu mt-haplotype and the remaining eight haplotypes clustered into the *B. taurus* clade (Bradley et al, 1996; Lai et al, 2005). Brah-1 occurred in duplicate, and the others occurred once each.

The accession number and cattle breeds of partial haplotypes from GenBank and 10 Brahman cattle are listed. J1 represents for Japanese black cattle, U87633; QC-06-A1 for Qinchuan cattle, AF514784; V00654 for Holstein cow; FR482, FR521, FR479 for Friesian, AY378146, AY378144, AY378145; CH-04-B1 for Chinese Holstein, AF516713; Lulu-1, Lulu-3, Lulu-4 for Lulu cattle (Nepal), AB085918, AB085920, AB085921; Hariana-1, Hariana-2 for Hariana (India), AB085922, AB085923; ON2770, ON2771, ON2774 for Ongole (India), AY378134, AY378135, AY378136; YY-03-G1 for Yueyang Yellow cattle, AY119678; Nellore/Nelore for Nellore (Brazil), NC_005971; TH982 for Tharparkar (India), AY318137; Zwergzebu for German small-typed zebu, AF492350; Brah-1 – 9 for Brahman sequenced in this study, respectively.

## 2.2 The phylogenetic status of Brahman cattle between *B. indicus* and *B. taurus*

Together with a range of complete D-loop sequences of the genus *Bos* published in GenBank, we aligned the polymorphic sites (Fig. 1). All the haplotypes were clustered into two distinct clades, the *B. taurus* and *B. indicus* clades (Fig. 2). The Brahman cattle carried both *B. taurus* and *B. indicus*

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                1111122 2222222222 2223333333 3333333333 3344444444 4455555556 7777777777 78888
               2253566602 3455566678 9990011222 2333344555 7901344556 6701112585 1123577889 90345
               6755002838 1918956763 1342818256 8014967026 4450912780 5440126674 4718401238 94160
V00654         TATTATCGAT GATCCAGCAT GCTGTGTTTG TGTATTTTAT TGG-ACCCCA TGTACGTGCT AAACT--TTC TCATG
FR482          C......... .......... .......... ........A. ...-...... .......... .....--... .....
Brah-5         .......... .......... .......... ........A. ...-...... .......... .G...--... .....
Brah-3         .......... .........C .......... ........A. ...-.....G .......... .....CC... .....
Brah-4         .......... .......... .......... ........A. ...-...... .A........ .G...--... .....
Brah-2         .......... .......... .......... ........A. ...-...... .......A.. .G...C-... .....
XZ-03-G3       .......... .......... .......C.. ........A. ...-...... .........C .G...C-... .....
Lulu-3         .......... .........C.. ....... C.......A. ...-...... .......... .G...C-... .....
Brah-1         .......... .......... .......... C.......A. ...-.T.... ..C....... .G...--... .....
Lulu-1         .......A.. .......... .......... ........A. ...-...... ....T..... GG...--... .....
CH-04-B1       .......... ......C... .......... ........A. .A.-.T.... C.......... .G...--... .....
QC-06-A1       .......... ..C....... ...A...... ........A. ...-...... .....A.... .G...--... .....
J1             .........C ..C....... ...A...... ........A. ...-...... .....A.... .G...--... .....
Brah-9         .......... A...T..... .......C.. ........A. ...-...... C.......... .G...--... .....
Brah-7         .......... A...T..... .T.....C.. ........A. ...-...... C.......... .G...C-... .....
FR479          .......... A...T..... .......C.. ........A. ...-...... C...T..... .G...C-... .....
FR521          .......... A...TG.... .......C.. ........A. ...-...... C.......... .G...C-... .....
Brah-8         .......... A...TG.... .......C.. ........A. ...-...... C.....C... .G.T.--... .....
Brah-6         .G..TCGAG. A..T..AT.C AT...ACCCA CACGCCC.-C ..AAG..TT. ...GT..A.C G.G.CC-CCT C..CA
Nellore        .G...CGAG. A..T..AT.C AT...ACCCA CAC.CCC.-C ..AAG..TT. ...GT..A.C G.G.CC-CCT C..CA
YY-03-G1       .G...CGAG. A..T..AT.C AT...ACCCA CAC.CCC.-C ..AAG..TT. ...GT..A.C G.G.C--CCT C..CA
TH982          .G...CGAG. A..T..AT.C AT..CACCCA CAC.CCC.-C ..AAG..TT. ...GT..A.C G.G.CC-CCT C..CA
Hariana-2      .G...CGAG. A..T..AT.C AT...ACCCA CAC.CCC.-C ..AAG..TT. ....T..A.C G.G.CCC.CT C..CA
ON2770         .G...CGAG. A..T..AT.. AT...ACCCA CAC.CCC.-C ..AAG..TT. ...GT..A.C G.G.CC-CCT C.TCA
ON2774         .G...CGAG. A..TT.AT.. AT...ACCCA CAC.CCCC-C ..AAG..TT. ...GT..A.C G.G.C--CCT C..CA
Lulu-4         .G...CGAG. A..T..AT.C A.C..ACCCA CAC.CCCC-C ..AAG.TTT. ....T..ATC G.G.CC-CCT C..CA
Zwergzebu      .G...CGAG. A..T..AT.C A.C..ACCCA CAC.CCCC-C ..AAG.TTT. ...GT..ATC G...CC-CCT C..CA
Hariana-1      .G.C.CGAG. A.....ATGC A.C..ACCCA CAC.CCCC-C ..AAG.TTT. ...GT..ATC G...CC-CCT C..CA
ON2771         .GC..CGAG. AG.TT.AT.C A.C..ACCCA CAC.CCCC-C C.AAG.TTT. ...GT..ATCG .G.CC-CCT CT.CA
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Fig. 1　The polymorphic sites of the mtDNA D-loop of Brahman cattle defined according to Anderson et al (1982) and published in GenBank

"·" indicates the same base pair, "-" indicates deletion/insertion.

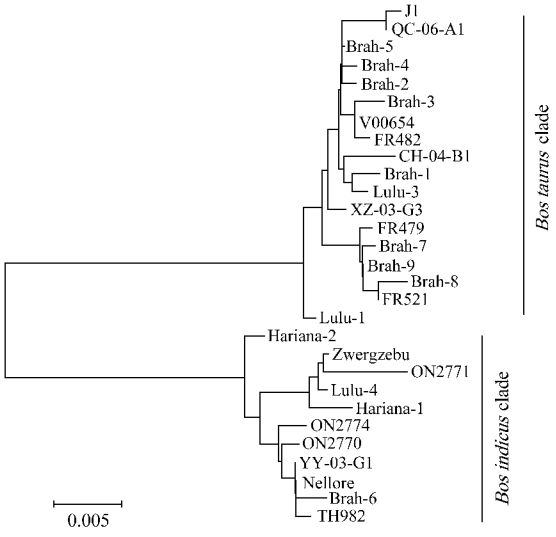


Fig. 2 A Neighbour Joined tree for Brahman cattle and GenBank data, constructed by Mega 2.1

mt-haplotypes, suggesting that Brahman cattle contained both *indicine* and *taurine* genetic background. Ninety percent of the mt-haplotypes were *B. taurus*. The eight mt-haplotypes of Brahman cattle were intermingled with the Lulu, Qinchun, Xizhen, Holstein and Japanese Brown branch. Only Brah-6 mt-haplotype belonged to the Asian zebu group, clustering with the Indian subcontinental zebu distributed in Nepal, India and Pakistan. However, zebu introgression occurred frequently; Lulu, Yueyang cattle and other taurine cattle breeds, showing typical morphological features of *B. taurus*, possessed *B. indicus* mt-haplotypes.

## 3 Discussion

The Brahman cattle showed high genetic diversity, of which haplotype diversity and nucleotide diversity were 0.978 and 1.43% respectively, congruent with previous results (Expected heterogeneity, $H_{exp}$ = 0.7396, Qu et al, 2006). Brahman cattle carried both *B. indicus* and *B. taurus* mitochondrial lineage, which is one of the leading reasons that Brahman cattle possess high genetic diversity (Loftus et al, 1999). Kikkawa et al (1995) and Meirelles et al (1999) found that nearly all Brahman cows that had no *B. indicus* mt-type resulted from male-mediated introgression. This may indicate that Brahman cattle is not a pure zebu breed because its taurine mt-haplotypes were 90% of the total. After the 1960's introduction of Indian Ongole cattle to Brazilian Nellore cattle, Nellore cattle were bred and as expected possessed Asian *B. indicus* mt-type, but *B. taurus* mt-types were still predominant at 79% (Meirelles et al, 1999; Oklahoma State University Board of Regents, 2000). Although the Butana and Kenana cattle in East Africa are characterized by zebu features, there was no evidence of the dispersal of African zebu with its hybrid origin in the Asian zebu clade (Loftus et al, 1994b). It seems that many people have underestimated the contribution of *B. taurus* during the formation of the zebu breeds. Modern zebu breeds have both *B. taurus* and *B. indicus* mt-types and they were bred through male-mediating and grading-crossing. The newly bred beef cattle breeds were improved in adaptability and productivity compared with the local breeds (Meirelles et al, 1999). Thus Brahman cattle were originally selected from the Indian subcontinent and spread widely in the tropical and subtropical areas, or hot and dry environments, of America and Africa. Mongolian cattle, with *B. taurus* morphological features, carried distinct *B. indicus* mitochondrial lineages (20%) and they didn't inherit the selective advantages of zebu cattle, such as heat tolerance, tick resistance and a distinct hump, but are well adapted to the northern cooler weather as northern cattle (Mannen et al, 2004). Most cattle are known to have hybrid origins and have undergone zebu introgression, such as Philippine native cattle (Kikkawa et al, 1995), Lulu (Fujise et al, 2003), Yunnan cattle (Yu et al, 1999), Qinchuan, Jinnan (Lei et al, 2004), Guanling (Liu et al, 2005) and Sichuan cattle (Lai et al, 2005). Through crossbreeding the BMY cattle contained both taurine and indicine matrilineages (Data not shown) just as Yunnan cattle did. The BMY breed is well adapted to the tropical and humid weather in South China and can still graze in snow. These economic characteristics have been stably inherited up to now (Wen et al, 2003; Yunnan Beef Cattle and Pasture Research Center, 2005 – 2006).

*Bos indicus* and *B. taurus* were clustered into two distinct clades (Fig. 2). Brah-6 was the closest to the Asian zebu and the other eight haplotypes were intermingled into the *B. taurus* clade, including Qinchuan, Xizhen, Holstein and Japanese Brown (Loftus et al, 1994a, b; Bradley et al, 1996; Mannen et al, 1998; Troy et al, 2001; Magee et al, 2002; Fujise et al, 2003; Lai et al, 2005). It is worth noting that there is a predominant taurine genetic background in most of today's zebu breeds except for the Asian zebu; *B. taurus* contributed more to today's zebu breeds

than the Asian zebu did to *B. taurus*. All the *B. indicus mt-haplotypes belonged to the Asian zebu lineage, and the Indian Hariana-2 was the proximal breed of the B. indicus* clade, indicating that Hariana-2 may be from one of the original breeds. We also found strong support that the zebu has undergone an independent domestication and probably originated from the Indian subcontinent (Loftus et al, 1994a; Bradley et al, 1996; Fujise et al, 2003).

Clarifying the breeding history of Brahman cattle was a complex problem as they have the genetic background of both the Asian zebu and most European beef cattle. This reflects the main contribution from the introduction of the Asian zebu to upgrade native beef cattle breeds to form the modern zebu breeds (Mannen et al, 2004; Henkes et al, 2005).

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## 本刊编委李庆伟教授简介

李庆伟教授

李庆伟, 男, 1955 年 5 月生于辽宁大连, 博士, 校一级特聘教授, 博士生导师, 细胞生物学方向学术带头人。1996 年毕业于南京师范大学生命科学学院细胞遗传专业, 获博士学位。1982 年开始在辽宁师范大学生命科学学院从事教学、科研工作。历任辽宁师范大学生物系副系主任、生命科学学院院长、校科研处处长、校长助理, 现任辽宁师范大学副校长。在学术方面, 担任辽宁省生物技术协会理事、辽宁省鸟类学会常务理事、辽宁省动物学会理事、辽宁省生态学会理事、辽宁省野生动物保护协会常务理事、中国遗传学会动物专业委员会委员,《遗传学报》、《遗传》、《动物学研究》等刊物编委。1993 年以来享受国务院政府特殊津贴, 1996 年首批入选国家人事部等七部委实施的“百千万人才工程”全省 32 人之一, 1997 获大连市劳动模范称号, 1998 年评为辽宁省青年专业技术拔尖人才, 1999 年获辽宁省优秀共产党员称号。

80 年代中期以来一直从事鸟类细胞和分子进化的研究, 先后完成了 280 余种鸟类染色体核型和部分种类的 G、C 带型的研究。首次提出鸟类染色体进化的“瀑布模型”, 是国内外同类研究鸟类染色体核型和发表论文最多的学术团队之一, 出版了国际上第一部《Chromosome Atlas of Birds》专著。90 年代初又率先开展了鸟类 mtDNA 分子进化的研究, 首次发现鸟类增大的 mtDNA(18 853 bp), 并首次将鸟类线粒体基因的 tRNA 二级结构引入鸟类种群特征的比较研究中, 取得了很好的结果, 在鸟类分子进化研究领域做出了原创性工作, 得到了国内外同行的高度评价。2000 年又率先在国际上开展了日本七鳃鳗基因组学和蛋白质组学的研究, 并对其重要功能基因和蛋白进行了生物制药的研发, 取得了具有创新性的研究成果。在李庆伟教授的倡导下, 2004 年 10 月成立了辽宁师范大学海洋生物功能基因与蛋白质组学研究所, 组建了一个优秀的学术创新团队, 积极地开展日本七鳃鳗功能基因组学、蛋白质组学与海洋生物制药开发的研究, 该科研团队入选 2006 年辽宁省高校创新团队支持计划。

1994 年以来李庆伟教授先后主持过 6 项国家自然科学基金项目, 1 项海洋“863”项目, 省市级项目 10 余项, 发表论文 80 余篇, 其中 SCI 收录 10 多篇。主编、参编学术专著 4 部, 近年获辽宁省政府科学二等奖 1 项, 三等奖 2 项, 申请专利 10 项。