

Phylogeny of Y chromosomes from bovine species

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Abstract

The Bovini tribe contains domestic and wild cattle-like species, several of which are cross-fertile. We present a completely resolved Y-chromosomal phylogeny, which is better in agreement with autosomal phylogeny, morphological data, cross-fertility and estimates of divergence times than mitochondrial data. The tree links *Bos grunniens* (yak) to *Bison*, so the commonly accepted *Bos* genus is not monophyletic. Therefore, we advocate the term *Poephagus* as designation of yak. This work illustrates the resolving power of Y-chromosomal variation for cladistic studies of closely related species.

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The tribe Bovini comprises the wild and domestic *Bos*, *Bison*, *Bubalus* and *Syncerus* species, which have adapted worldwide to various climates. *Bos* and *Bison* species have diverged about 1 Myr or less (Janecek *et al.*, 1996; Buntjer *et al.*, 2002). Female hybrids of these species are fertile as are the male hybrids from the more recently diverged ox (*Bos taurus*) and zebu (*Bos indicus*), gaur (*Bos gaurus*) and gayal (*Bos frontalis*), and American (*Bison bison*) and European (*Bison bonasus*) bison, respectively (Lenstra and Bradley, 1999). Hybridization of bovine species occurs worldwide either spontaneously or by breeding and has led to the occurrence of non-specific mitochondrial DNA (Nijman *et al.*, 2003; Verkaar *et al.*, 2003). Hybridization may also have played a part in the bovine speciation, complicating the resolution of the bovine phylogeny (Buntjer *et al.*, 2002). Verkaar *et al.* (2004) found that the American and European bison or wisent had similar Y chromosomes, but well-diverged mitochondrial DNA. The separate maternal origins of the two bison species were supported by high bootstrapping values (see also Janecek *et al.*, 1996; Hassanin and Ropiquet, 2007) and led to the proposal that wisent has a hybrid origin. However, the resolution of the Y-chromosomal phylogeny (Verkaar *et al.*, 2004) was

still incomplete. Here we report a comparison of 4685 bp of single-copy Y-chromosomal sequences. We arrive at a completely resolved phylogeny that is in better agreement with a partially resolved autosomal phylogeny (Buntjer *et al.*, 2002), morphological resemblance and hybrid fertility than the mitochondrial phylogeny.

Methods

Origin of samples, DNA extraction and polymerase chain reaction conditions have been described previously (Verkaar *et al.*, 2004). Primers for amplification and sequencing 416 bp from the first *DBY* intron, 1187 bp from the *ZFY* intron 10–exon 11 junction, 507 bp *ZFY* mRNA and 2797 bp containing the *SRY* gene, respectively, are listed in Table 1. Sequencing from both ends was done with 75 ng polymerase chain reaction product, the Cy5 Big Dye terminator kit (Applied Biosystems, Foster City, CA) and an ABI Prism 310 or 3700 Sequencer. GenBank accession codes for the alignments are DQ336526–DQ336565, respectively. Phylogenetic analyses were performed by the PAUP 4.0 package. Maximum likelihood trees were reconstructed on the basis of the HKY model. Bayesian trees were reconstructed by using the program MrBayes (Huelsenbeck *et al.*, 2000).

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Table 1
Oligonucleotide primers for amplification and sequencing of Y-chromosomal DNA segments

GenBank entry	Forward (5'–3')	positions	Reverse (5'–3')	positions
ZFY1	AF032366 CAGGTGAGGGCACATGAG *GATCTCAGAGGAAATCCAGTA	1–15 41–62	ATCACATTCGATGGCCTT *GATTGTGTAACCTTATCTTCTT	1022–1004 969–948
ZFY2	AF032366 ATATGCTTGAAGAGACGACAAC *TTCATTGGATCACTTATGCTC	715–738 744–764	AGTCAGAAGACAAATGTCACA *TATGGATTTCGATGTGCTT	1827–1807 1699–1681
ZFY3	AF032867 TTCTAATTGAAGACGCATGTG *AAGTGTGACATTTGTCTTCT	1752–1773 1804–1823	CAACTTCTTTATGGTGTCTGTG *TCTTCGGAACCCCTTTCTTG	2395–2376 2349–2331
SRY3	AF026566† AGCCTTTGAAGTTTCTACTGTC *ACAATATACAGTTGTTACTTCCA	3584–3605 3765–3788	CCCCAATACCTCCCTCAATAC *CAGAATTTGTGAATGTGC	4764–4743 4662–4645
SRY4	AB039748 GTCTGCTGCACCTTCATC *AAATAACTTCATAATGACACTT	701–718 738–759	CTTATTGTGGCCAGGCTTGTC *TGTGACCGGCTTAATTGGCTT	1669–1648 1596–1576
SRY5	AB039748 CCGGGCTATAAATATCGACCTC *CCACAGAAATCGCTTCTGTC	1421–1441 1460–1479	GATGAAACCTTGGGTCTCACAG *TATGACTAAAGGAACTGAG	2483–2462 2395–2377
SRY6	AB039748 CTCACAAATTCATGGTACAGAG *TAAAAAATCTCGTACTTGGGA	2150–2171 2172–2191	GATCTTGTTTATCCCATCCC *GCCACATAATCTGAATAAT	2647–2629 2624–2606
DBY12‡	AY928816 AGCAGTTTGGRTCTCGWGA *GTGTGTATAATATAGSATTTTCAG	22–45	CCAACGACTATGWCCACT *GTAACCTTCAAAGATGCTAGT	536–516
M13 tail	TGAAAACGACGGCCAGT		AGGAAACAGCTATGACCAT	

*M13-specific extensions.

†Sheep homologue.

‡From Hellborg and Ellegren (2003).

Results

We sequenced 408 bp of *DBY*, 1178 and 507 bp of *ZFY* and 2768 bp of *SRY* sequence, respectively, as counted for the *Bos taurus* sequence, 3030 of which are non-coding. Dismissing all indels and regions with missing data yielded an alignment of 4685 bp for 10 bovine species.

The divergence of the Y-chromosomal sequences of two different species is typically in the range of 0.01–0.03 per nucleotide position (Fig. 1) with hardly any difference between coding and non-coding regions (not shown) and

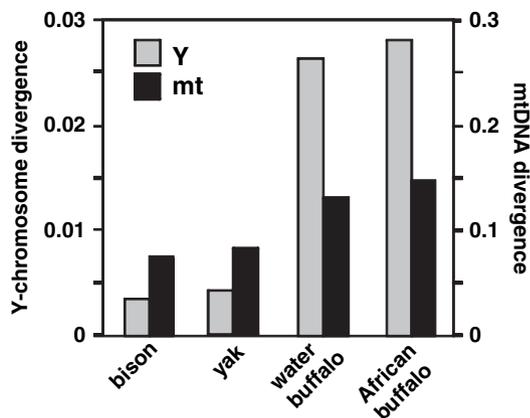


Fig. 1. Divergence (differences per nucleotide position) of the ox and zebu Y-chromosomal and mitochondrial (Verkaar *et al.*, 2004) sequences, respectively, with the corresponding sequences from the indicated species. Values were averaged over the values for ox and zebu. Similar trends were observed if comparisons were made with coding or non-coding Y-chromosomal sequences or with mitochondrial non-coding transversions.

clearly lower than the divergence of mitochondrial DNA. However, a striking difference with the mitochondrial sequence data is an apparent lack of saturation. While for mitochondrial DNA (Verkaar *et al.*, 2004) the sequence divergence of mitochondrial sequences of ox or zebu and the buffalo species is less than twice the divergence of ox or zebu with bison or yak, the ratio for Y-chromosomal DNA is in the range 6–8. This is in good agreement with estimated divergence times of about 1 Myr for taurine cattle and bison or yak and 5–10 Myr for taurine cattle and buffalo species.

Tree reconstructions by Neighbor-Joining, Maximum Parsimony, Maximum Likelihood and Bayesian reconstruction all give the same topology with satisfactory bootstrapping values. As reported before, a clustering is observed of the Y chromosomes from the American and European bison, which are completely cross-fertile despite the divergence of their mitochondrial DNA sequences (Verkaar *et al.*, 2004; Hassanin and Ropiquet, 2007). However, the present data also indicate a clustering of the South-east Asian banteng, gaur and gayal, and of zebu and cattle, respectively. At a deeper level, banteng, gayal and gaur are linked to ox and zebu.

Discussion

Evolution of Y chromosomes

Y chromosomes combine relatively low diversity within species (Hellborg and Ellegren, 2004; Brändli *et al.*, 2005) with a relatively high divergence on the species level (Lawson and Hewitt, 2002; Hughes *et al.*, 2005). Divergence is proposed to be caused by hitch-

hiking during positive selection acting on few of the Y-chromosomal genes (Hughes *et al.*, 2005) or by sexual selection (Roldan and Gomendio, 1999). Selection is probably intensified by sperm competition (Hughes *et al.*, 2005), the state of hemizyosity and possibly also by genetic conflicts (Hurst *et al.*, 1996). Within species, selective sweeps are often facilitated by low male population sizes and lead to homogenization of Y-chromosomal sequences.

This combination of factors may explain why the ratio of male to female mutation rate depends on the taxonomic level (Sandstedt and Tucker, 2004) and at the same time makes the Y chromosome attractive for cladistic studies (Johnson *et al.*, 2006). Sequence divergence is most pronounced at higher taxonomic levels by an apparent lack of saturation. In addition, tree reconstructions are not confounded by recombination as has often been observed with autosomal genes. Finally, the paternal transmission of the Y chromosome is especially informative for the detection of interbreeding between species. This has been demonstrated in ruminants (Cathey *et al.*, 1998; Nijman *et al.*, 2003; Pidancier *et al.*, 2006; Ropiquet and Hassanin, 2006; Verkaar *et al.*, 2004), in *Camelidae* (Kadwell *et al.* 2001) and in primates (Tosi *et al.*, 2000), while a hybrid origin has also been suspected for the extinct kourprey cattle (Hassanin and Ropiquet, 2007). Emergence of new species by interspecific hybridization would be a non-canonical “transpatric” mode of speciation (Verkaar *et al.*, 2004).

Bovine phylogeny

Our data allow a consistent reconstruction of the phylogeny of the bovine species on the basis of nuclear

DNA, which completes previous topologies on the basis of morphology (Geraads, 1992), microsatellites (Ritz *et al.*, 2000), amplified fragment length polymorphism (AFLP) (Buntjer *et al.*, 2002) and mitochondrial DNA (Verkaar *et al.*, 2004; Hassanin and Ropiquet, 2007).

Y-chromosomal DNA as well as mitochondrial DNA indicate a close relationship between the semidomestic gayal (or mithun) and the wild gaur. Recently, it was proposed that gayal and gaur are different species (Ma *et al.*, 2007). However, this was based on a comparison with gaur mitochondrial cytochrome *b* sequences (entries DQ459330 and DQ459331 from GenBank) that evidently originated from zebu.

Apart from the anomalous origin of wisent, mitochondrial data (Fig. 2; Verkaar *et al.*, 2004) were not conclusive about the relationships between the clusters of bison and yak, banteng, gayal and gaur, and ox and zebu, respectively. Our data indicate that banteng, gayal and gaur, often denoted as *Bibos* rather than *Bos* species, are clustered with *Bos taurus* and *Bos indicus*. However, yak is commonly denoted as *Bos grunniens* and *mutus* for the domestic and wild forms, respectively, but has a separate position. Remarkably, mitochondrial (Verkaar *et al.*, 2003) and AFLP (Buntjer *et al.*, 2002) data clustered yak with bison. However, both topologies imply that the current *Bos* taxon is not monophyletic. Therefore, the molecular data support a nomenclature that denotes yak as *Poephagus grunniens* or *Poephagus mutus*.

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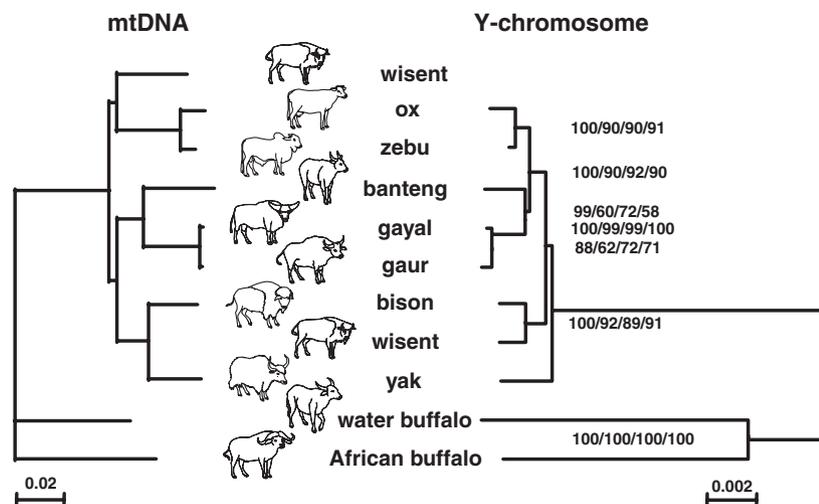


Fig. 2. Mitochondrial and Y-chromosomal phylogeny of bovine species. The mitochondrial Neighbor-Joining tree is from Verkaar *et al.* (2004). The values near the Y-chromosomal nodes indicate statistical support of MrBayes (percentage of times the clade occurs during sampling of trees), maximum likelihood, parsimony and Neighbor-Joining (bootstrap percentages after 1000 replications) algorithms, respectively. The relative branch lengths are those of the Neighbor-Joining tree.

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