

# Taurine and zebu admixture in Near Eastern cattle: a comparison of mitochondrial, autosomal and Y-chromosomal data

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## Summary

Eight *Bos taurus* cattle breeds from the Near East region were screened with a *Bos indicus* (zebu)-diagnostic Y-specific microsatellite (*INRA124*) to estimate the proportion of zebu Y chromosomes in each population. This value was compared with previously published values for zebu introgression for both the mitochondrial and autosomal gene pools of the same breeds. All breeds revealed considerable levels of introgression from *B. indicus* cattle when the autosomal data were taken into consideration; this was particularly apparent in cattle populations from Iraq in the east, and declined in the populations further west towards Anatolia. This non-random pattern of introgression and admixture is suggestive of the introduction of zebu cattle from the region corresponding to present-day Iran and northern Pakistan. In addition, the maternal and paternal markers demonstrate that the movement of cattle into and within the Near East was complex.

**Keywords** admixture, cattle, introgression, microsatellite, mitochondria, Y chromosome.

The two principal taxonomic groups of domestic cattle are *Bos taurus* (termed taurine cattle) and *Bos indicus* (termed zebu cattle). Archaeological studies (Allchin 1969; Grigson 1980; Meadow 1984, 1993) and DNA-based genetic surveys (Bradley *et al.* 1994, 1996, 1998; Loftus *et al.* 1994; MacHugh *et al.* 1997) have demonstrated that the two subspecies arose through separate domestication events. Taurine animals in the Near East represent the first herds domesticated some 10 000 BP, with the earliest evidence dating to approximately 10 650–10 250 cal. BP from Dja'de el Mughara in the Middle Euphrates Valley (Helmer *et al.* 2005). Zebu cattle, on the other hand, were first domesticated in the early Neolithic cultures of Baluchistan (Naik 1978; Meadow 1984, 1993). Clear molecular differences exist between the zebu and taurine genomes that can be used to detect gene flow and introgression between the two taxa (Bradley *et al.* 1994, 1996; Loftus *et al.* 1994; MacHugh *et al.* 1997; Edwards *et al.* 2000; Hanotte *et al.* 2000, 2002; Troy *et al.* 2001; Kumar *et al.* 2003). It has been shown that domestic cattle can display marked sex-

biased admixture and migration patterns, for example, the zebu genome has spread across Africa through male-mediated gene flow (MacHugh *et al.* 1997; Bradley *et al.* 1998; Hanotte *et al.* 2000, 2002; MacHugh & Bradley 2001). This has produced asymmetrical distributions of the maternal mitochondrial DNA (mtDNA) and paternal Y-chromosomal components, with the autosomal genome representing an intermediate picture between these two uniparental extremes. Analyses of data from biparental autosomal markers has demonstrated that the Middle Eastern region represents a complex hybrid zone between the two subspecies (Loftus *et al.* 1999).

In the present study, eight populations from Anatolia, the Middle East and Egypt were analysed with a zebu-diagnostic Y-specific microsatellite (*INRA124*) to estimate the proportion of zebu Y chromosomes in each population. This value was then compared with previously published values for zebu introgression into both the mitochondrial and autosomal gene pools of the same breeds (Table 1). The results showed evidence of extensive zebu introgression into Near Eastern cattle, indicative of widespread introductions, and perhaps selective breeding of cattle for adaptability to changes in the local climate.

DNA samples were analysed from a subset of 338 animals previously described by Loftus *et al.* (1999) and Magee *et al.* (2007). The sample panel represented eight distinct cattle breeds originating from four countries in the Middle East and Anatolia: Anatolian Black (AB), Damascus (DA), East

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**Table 1** Zebu–taurine admixture for uniparental and autosomal genomic systems.

Cattle population <sup>1</sup>	Code	Breed origin	Mitochondrial data		Autosomal data		Y-chromosome data	
			Sample size	Zebu mitotypes (%)	Sample size	Zebu admixture (%)	Sample size	Zebu chromosomes (%)
Turkish Grey	TR	Western Turkey	11	0	43	14	21	0
Anatolian Black	AB	Central Turkey	12	0	41	35	14	0
South Anatolian Red	SA	Southern Turkey	11	0	43	23	7	0
East Anatolian Red	EA	Eastern Turkey	10	10.0	44	28	6	0
Damascus	DA	Syria	12	0	41	34	14	0
Egyptian	EY	Egypt	11	0	40	36	8	0
Kurdi	KU	Northern Iraq	12	16.7	37	39	5	20.0
Iraqi North	IN	Northern Iraq	20	20.0	–	–	12	66.7
Iraqi Central	IC	Central Iraq	13	23.1	–	–	10	30.0
Iraqi South	IS	Southern Iraq	16	43.8	–	–	7	85.7
Iraqi Total	IT	Iraq	–	–	42	34	–	–
Total			128	–	331	–	104	–
Average (%) zebu			–	11.4	–	30.4	–	20.2

<sup>1</sup>Cattle populations included in the analysis are shown with their country of origin, the number of individuals sampled and the associated two-letter codes. The percentage of zebu introgression was estimated using the criteria described in the text. Mitochondrial data were taken from Troy *et al.* (2001) and Magee *et al.* (2007), and autosomal admixture data from Freeman *et al.* (2006a).

Anatolian Red (EA), Egypt (EY), Iraq (IT), Kurdi (KU), South Anatolian Red (SA) and Turkish Grey (TR) (Table 1). In the case of the mitochondrial and Y-chromosomal data, the Iraqi breed was split into Northern (IN), Central (IC) and Southern (IS) subsamples. These breeds have been conventionally designated as *B. taurus*, exhibiting typical taurine morphology, with the exception of the Damascus-type cattle that display zebu characteristics such as a neck hump and dewlap (Felius 1995).

The mitochondrial haplotypes used in this study were published by Troy *et al.* (2001) and Magee *et al.* (2007). DNA sequences were determined from the D-loop control region, between positions 16 023 and 16 262, in a total of 128 animals. Individuals were assigned as zebu or taurine depending upon their similarity to either the main zebu mitotype or one of the four European taurine mitotypes as described by Bradley *et al.* (1996), and zebu/taurine proportions were calculated accordingly (Table 1).

The autosomal data presented here have been published previously (Loftus *et al.* 1999; Hanotte *et al.* 2002). DNA samples were collected from a total of 331 animals and the breeds were screened with a panel of six microsatellites (*ETH10*, *ETH152*, *ETH225*, *HEL13*, *ILSTS005* and *INRA005*) that displayed alleles diagnostic of zebu ancestry. Zebu population admixture proportions were estimated using these population-associated alleles (Freeman *et al.* 2006a) as shown in Table 1.

*INRA124*, a Y-specific microsatellite locus isolated by Vaiman *et al.* (1994), was genotyped in 104 male cattle from the Near East. This marker, previously reported by Hanotte *et al.* (1997), differentiates the taurine and zebu Y-chromosome types. Radioactively labelled PCRs were

carried out as described by Edwards *et al.* (2000). The samples were scored for the presence of either the 130-bp ‘zebu’ allele, or the 132-bp ‘taurine’ allele, and the proportion of zebu Y chromosomes was estimated for each breed (Table 1).

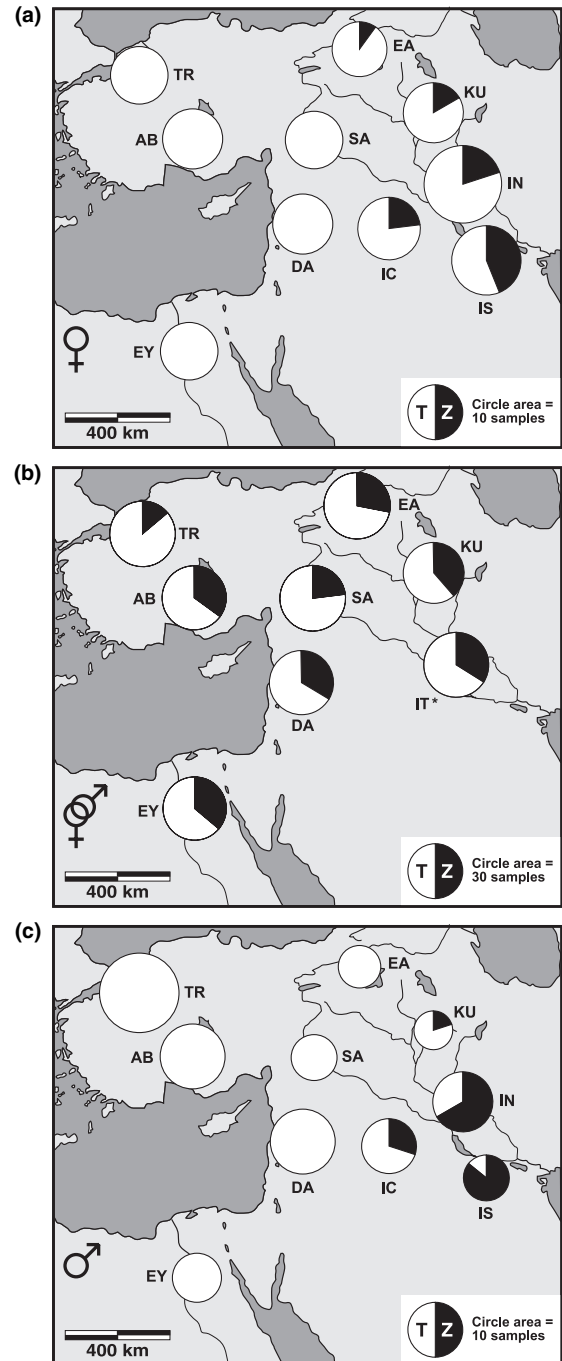
A summary diagram of zebu genetic introgression for the three genetic systems is illustrated in Fig. 1. The uniparental mtDNA and Y-chromosome zebu haplotypes were geographically distributed and showed a gradual decrease with increased proximity to Africa and Europe. The clinal nature of the zebu genetic introgression is clearly evident, with the non-random pattern suggestive of the introduction of zebu from the east. Unlike the situation observed across much of Africa, where the total absence of any zebu mitotypes in the African zebu populations studied to date reflects a clear history of male-mediated zebu introgression (Bradley *et al.* 1994, 1996; MacHugh *et al.* 1997; Hanotte *et al.* 2000), it appears that zebu mtDNA haplotypes have introgressed more readily into the Near East. This introgression may have occurred because of a relative lack of geographic barriers in the region, with the opportunity for whole-scale migrations of zebu herds including females.

The autosomal zebu admixture evident from the microsatellite data in these populations is markedly less discordant and is comparable with patterns of autosomal admixture observed in hybrid cattle from Africa (Hanotte *et al.* 2002). The eight populations represented herein have broadly similar proportions of autosomal zebu introgression with a mean of 30.4% and a range of 14–39%. In the population samples analysed here, the zebu mtDNA haplotype and the zebu Y chromosome were not evident in the Anatolian, Syrian or Egyptian populations. Other popula-

tions that have similarly distorted genome compositions (i.e. no zebu mtDNA, significant autosomal zebu levels and sparse zebu Y-chromosomal DNA) include the African Sanga and Kuri cattle (Hanotte *et al.* 2000, 2002; Troy *et al.* 2001; Freeman *et al.* 2004). In contrast, the Nellore cattle of Brazil display a high frequency of taurine mtDNA (Magee *et al.* 2002), very high levels of autosomal zebu DNA (Loftus *et al.* 1999; Kumar *et al.* 2003) and a 100% frequency of zebu Y chromosomes (Edwards *et al.* 2000), perhaps indicative of maternal taurine introgression into a zebu population.

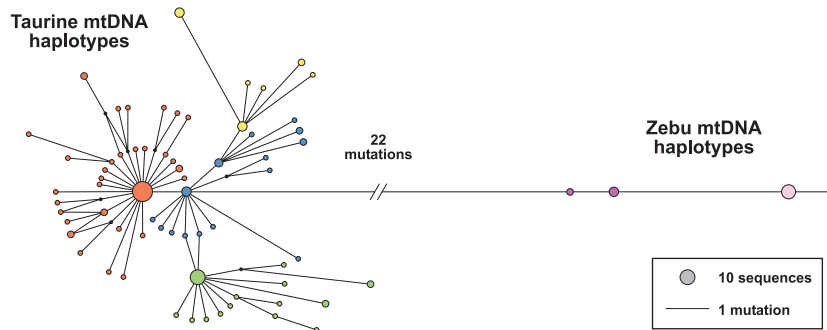
One explanation for the observed distribution of uniparental and biparental genetic variation in Middle Eastern cattle is that there was a metapopulation of wild aurochs in this region prior to domestication, which gave rise to both taurine and zebu cattle. This hypothesis can be discounted on a number of grounds and is discussed more fully by Bradley & Magee (2006). A reduced median phylogenetic network was constructed (Fig. 2) for the 128 Middle Eastern and Anatolian mtDNA sequences using 240 bp of the control region (Troy *et al.* 2001; Magee *et al.* 2007). This mtDNA phylogeny of cattle shows two clearly distinguishable taurine and zebu clades. If an ancient panmictic metapopulation of taurine and zebu cattle existed, present day mtDNA phylogenies would be more diverse, complex and 'bushy' (MacHugh & Bradley 2001). In addition, the Middle East represents the centre of diversity for taurine mtDNA (Troy *et al.* 2001), but the 16 zebu mtDNA haplotypes in this region show a paucity of sequence variation, indicative of a founder effect because of genetic introgression.

The asymmetrical genome composition of Near Eastern cattle populations may be better explained by a model that includes a dynamic 'ebb and flow' scenario of taurine/zebu admixture. The demographic pattern observed today represents a mixture of ancient indigenous cattle and animals introduced by migrants from the adjacent areas of Africa, Europe and the Indian subcontinent over the 10 000 years since domestication occurred in this region. It is possible to imagine a scenario whereby the original early domesticated mtDNA, autosomal and Y-chromosome pools were essentially taurine, and were then subjected to substantial introgression from zebu populations over time. This scenario is supported by analyses of linked microsatellite markers on the bovine X chromosome, which indicate that the age of zebu–taurine admixture in the Near East is relatively ancient (Freeman *et al.* 2006b). It is currently difficult to date the onset or maxim of this influx; however, archaeological evidence suggests the presence of zebu cattle in Jordan dating to 3400 BP (Clason 1978). It is therefore possible that the significant cline of zebu influence across the Near East reflects the influence of ancient trade links, such as the silk route. In addition, the mid-Holocene (8000 to 3000 BP) was a time of substantial climatic variability (Sandweiss *et al.* 1999), and there is environmental evi-



**Figure 1** Maps showing the relative proportions of zebu admixture in the three genomic components (a, mitochondria; b, autosomes; c, Y chromosome) from cattle populations across the Near East. Sample size is proportional to the area of the pie-circles indicated on each of the three diagrams, and the two letter codes are detailed in Table 1. Admixture proportions were estimated using the percentage zebu alleles (see text for further explanation), with the black portion of the circles corresponding to the values in Table 1. \*In the case of the autosomal data, the Iraqi breed (IT) was analysed as a total sample.

dence that Mesopotamia suffered a prolonged drought around 4000–3000 BP (Neumann & Parpola 1987). Consequently, it has been suggested that ancient herders may



**Figure 2** Partially reduced median phylogenetic network constructed for 128 Middle Eastern and Anatolian mitochondrial DNA (mtDNA) sequences (see text for further explanation). Circles represent sequence haplotypes; the areas of the circles are proportional to the frequencies of the haplotypes. Black points are theoretical intermediate nodes. Branches between haplotypes represent mutations and are all to scale, except for the 22-mutation branch between the central taurine and zebu clusters. The network was reduced, where possible, at positions previously determined as hypermutable by Troy *et al.* (2001). The colour-coding scheme for the taurine mtDNA haplogroups was followed according to Troy *et al.* (2001).

have introduced significant numbers of arid-adapted zebu populations into the Near East at this time (Matthews 2002). In addition, Loftus *et al.* (1999) have speculated that the Islamic civilizations in the region may have favoured zebu-type cattle because of their links with Islamic culture and religion.

Zebu gene flow into the Near East would undoubtedly have been male-mediated to some extent and rapid dispersal of the zebu Y chromosome (MacHugh *et al.* 1997) would imply that even the Anatolian populations may have had zebu Y-chromosomal input at some stage. This would have increased the autosomal zebu admixture, but not necessarily the proportion of zebu mitochondria, as this genomic component tends to be more genetically inert, with a sex-biased dispersal. It is likely that there have been additional waves of zebu migration into the area due to, for example, the Arab expansions of the 7th century AD (Hourani 1991). If this scenario of zebu population movements is correct, substantial introgression of taurine cattle must have taken place since the last great migration of zebu into the Near East. There could have been whole-scale introgression of taurine populations to the extent that the zebu Y chromosome has been eliminated in the west or perhaps deliberate use of taurine bulls within the same period, possibly by recent upgrading with male European genetic material. Alternatively, gradual local homogenization of disjunct taurine populations over time may account for present-day patterns of variation in the three genetic systems.

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