

Neolithic cattle domestication as seen from ancient DNA

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INTRODUCTION

THE EARLY NEOLITHIC comprises the time when pre-farming people became sedentary and subsequently began to domesticate plants and animals. The first settlers appeared about 12,000 years ago in the Middle and Near East; the Neolithic then expanded all over Europe from about 7000 cal BC onwards. The question is: did the first agro-pastoralists move to Europe, together with their plants and animals, or was it rather a cultural transfer where the Mesolithic people of Europe adopted the idea of domestication? It is possible that animals were imported without major human migration. We know that many plant species, and some animal species, at least sheep and goat, were imported from the Near East, as no wild progenitors existed in Europe. With regard to domestic cattle (*B. taurus*), however, the situation is different. Its wild ancestor is the aurochs (*B. primigenius*), which was prevalent all over Europe, Asia and North Africa. Therefore the European aurochs remains a potential progenitor of northern cattle breeds. Even if all cattle were imported, it is still possible that crossbreeding occurred. This could have happened either purposefully (for example, through young female aurochs being caught) or unwillingly (for example, when herds were driven to the forests and the cows could not be kept separate from wild bulls).

The most up-to-date knowledge of cattle domestication is the achievement of archaeological and archaeozoological studies. The morphological methods are based on size differences, with domesticated animals usually being smaller compared to their wild relatives. These measurements are sometimes insecure due to sexual dimorphism, high fragmentation of bones, age or the nutritional status of the animals. Morphological methods are limited in the way that these data cannot tell the relation between populations or reveal hybrids. This information can only be received from molecular genetic data.

Studies on modern cattle populations already demonstrate the relations of the two major cattle breeds, the humpless taurine cattle (*B. taurus*) and the Asian humped zebu (*B. indicus*). Studies by Loftus *et al.* (1994), Bradley *et al.* (1996) and MacHugh *et al.* (1997) showed that these two groups stem from independent domestication events in different geographical regions. Concerning the taurine cattle, recent population studies show that today the genetic diversity is highest in the Near and Middle East (Loftus *et al.* 1999; Troy *et al.* 2001). This is an indication of the centre of origin in this region. But modern data can be biased by recent breeding practices and introgression. Only the analysis of ancient samples can help to get at detailed information about prehistoric situations. In this study, we present ancient DNA data from mainly Neolithic bones of both cattle and aurochs from across Central and Eastern Europe.

MATERIALS AND METHOD

Samples and amplified loci

Altogether 161 ancient bones were analysed. The geographic distribution covers France, Germany, the Balkans and the Near East. Samples are mainly Neolithic but some are dated to the Mesolithic and Bronze Age. Information about origin, age, morphometric classification and haplotypes is given in Table 1.

The analysed locus is the HVR I region within the mitochondrial d-loop. The mitochondrial genome is only maternally inherited and does not recombine. Therefore the maternal lineage can be traced back for many generations as changes only occur by mutation. The d-loop is a non-coding region and the lack of selection enables mutations to accumulate at a high rate and therefore the HVR I region is very variable. It is also a prevalent marker for population genetic studies and a large modern dataset for comparison is available. Another advantage of using a mitochondrial marker is the high copy number of the mitochondrial genome. Each cell usually contains only one nucleus but up to several thousands of mitochondrial genomes. This increases the probability of finding sufficient DNA for a successful amplification within ancient samples.

In addition, the mitochondrial cytochrome *b* was amplified from two taurine and two aurochs samples. This marker is a gene and its sequence can be translated into the encoded amino acid sequence, which is mainly of interest for authentication of ancient DNA data.

Table 1. Archaeological sites, sample names, age, origin, classification and haplotypes for samples used in this study. Haplotype names refer to Fig. 1b. B.p.= *Bos primigenius*, B.t. = *Bos taurus*, Bison b. = *Bison bonasus*, * = independent replication in Dublin. ** These Near Eastern samples are replications and were first sequenced by C. J. Edwards at Trinity College, Dublin.

Archaeological site, Laboratory code	Archaeol. code	Date	Given by	Skeletal element	Species		Haplo-type
					Morphol.	Genetic	
Abu Gosh, Israel ABU 2	L146 level2, B1412 Hat. 696.16-696.10	PPNB	L. Kolska Horwitz	Scapula	B.p.	–	–
Albertfalva, Hungary		Bronze Age, Bell Beaker, 2500 BC	Alice Choyke				
ALB 1	65 obj 777 (small)			–	–	B.t.	T3
ALB 2	65 obj 777 (big)			–	–	B.p.	Pg
ALB 3	296 obj 3115			–	B.p.	B.t.	T3e
ALB 4	62 obj 1246			–	–	B.p.	Pg
Allendorf, Germany ALL 1	416/69	12030-52 cal. BP	N. Benecke	Radius	Bos spec.	B.p.	Pc
Asagi Pinar, Turkey		Karanovo IV	H. Hongo, M. Özdoğan				
AP 6	25-AP'97 131 18			–	B.t.	B.t.	T3c
AP 7	26-AP'99 9R 136			–	B.t.?	B.t.	Tc
Aswad, Syria			C. Edwards, J.-D. Vigne				
SYR 09	A375, 92 ADN ZV 120 ZZ124 h. 176 (A208)	Early EPPNB		–	B.p.	–	–
Atilit Yam, Israel YAM 4	Bld. 11 Hat.190-210	PPNC	L. Kolska Horwitz	Tibia	B.p.?	–	– (Continued)

Archaeological site, Laboratory code	Archaeol. code	Date	Given by	Skeletal element	Species Morphol.	Genetic	Haplo-type
Bad Abbach, Germany		Neolithic	G. Roth				
KOEL 1	151	4800–4650 BC		Radius	B.p.	–	–
KOEL 2	23-1			Humerus	B.t.	–	–
Berettyószentmárton, Hungary		Neolithic	István Vörös				
BER 1	56.11.186			–	–	–	–
BER 2	56.11.426			–	–	–	–
BER 5	56.11.553			–	–	–	–
BER 6	56.11.979			–	–	B.t.	T3
BER 8	55.4.132	Late Neolithic		–	–	–	–
Berlin, Germany		Medieval	Norbert Benecke				
WIB	–			–	Bison b.	Bison b.	
Budapest, Hungary		Iron Age	István Vörös				
WIB 1	–			Rib	Bison b.	Bison b.	
WIB 2	–			Rib	Bison b.	Bison b.	
Cave à L'ours, France		3694 BC cal.	Louis Chaix				
CAT 1	CP / 33.116			Skull	B.p.	B.p.	Pe
Catal Höyük, Turkey		7000–6000 BC	L. Martin, C. Edwards, J.-D. Vigne				
CH 02	CH1996#4			Metacarpus	Bos sp.	–	–
CH 03	CH1996#3			Metacarpus	Bos sp.	–	–
CH 04	CH1996#1			Metacarpus	Bos sp.	–	–
CH 11**	CH1996#X1			Metacarpus	Bos sp.	B.t.	T

Cayönü, Turkey	PPNB, 7000 BC	H. Hongo, M. Özdoğan				
CO 1	19 1991 30M 5-13 R			Bos sp.		
CO 2	2814 1987 25L 2-39 Lr			Bos sp.		
CO 5	87 27M 4-27 G					
CO 7	1991 29M7-15 R2			Bos sp.		
CO 9	2372 87 20L 9-46 CH2					
CO 13	91 30M 5-13 R				B.t.?	
CO 14	2672 1991 EF 7-6 Lr1			B.t.?		
Chateaux, d'Oex, Switzerland	ca. 300 BP	L. Chaix		Bos sp.	B.t.	T3
CAD 1						
Derenburg-Steinkohlenberg, Germany	Bernburg, 3600 BC	Hans-Jürgen Döhle				
DER 1	HK 87:183i		Tibia, distal	B.t.	B.t.	T3
Didi Gora, Georgia	Bronze/Iron Age	H.-P. Uerpmann				
DID 1	DG 85-3-46		Radius	Bos sp.		
DID2	DG 85-3-11		Calcaneus	Bos sp.		
DID 3	DG 85-3-10		Calcaneus	Bos sp.	B.t.	Tc
Dja'de, Syria						
SYR 01	A367, 92 ADN					
	B x4 F2 (A200)					
SYR 06	A372, 92 ADN					
	B x3 F2 (A205)					
Eilsleben, Germany	5000 BC, LBK	Hans-Jürgen Döhle				
EIL 1	HK 83:1040 I		Metacarpus	B.t.	B.t.	T3b
EIL 2	HK 88:354k		Humerus	B.t.	B.t.	T3
EIL 4*	HK 83:933 c		Humerus, distal	B.p.	B.p	P1
EIL 5	HK 83:754 o		Radius, distal	B.p.	B.p.	Ph
EIL 6	HK 83:702 i		Metacarpus, proximal	B.p.	B.p.	Ph
EIL 7	HK 85:142			B.t.	B.t.	T3

(Continued)

Archaeological site, Laboratory code	Archaeol. code	Date	Given by	Skeletal element	Species Morhpol.	Genetic	Haplo-type
EIL 8	HK 78:169			–	–	–	–
EIL 9	HK 85:138 p			Metacarpus, distal	B.t.	B.t.	T3
EIL 12	HK 78:162			–	B.t.	B.t.	T3
EIL 13	HK 88:487 g			Tibia, distal	B.t.	B.t.	T3
EIL 14	HK 83:928 I			Tibia, distal	B.p.	B.p.	Pf
Emmeloord, Netherlands							
EMM	J97 A/B	Late Neolith. – Bronze Age	L.P. Louwe Kooijmans	Skull	B.t.	B.t.	T3b
Etival, France							
ETI 1	?	Mesolithic, 5464 +/- 78 BC	Louis Chaix	–	B.p.	B.p.	P
Fikirtepe, Turkey							
FT 1	10-FT 700	6200–5500 BC	H. Hongo, M. Ödögan	–	Bos sp.	–	–
FT 4	13 FT-137			–	Bos sp.	–	–
FT 5	14 FT 19/20			–	Bos sp.	–	–
Goddelau, Germany							
GOD 1	GO 73F-2 90	Oldest LBK	Hans-Peter Uerpmann	Femur	–	B.t.	T3
GOD 2	GO 9-217			Pelvis	–	B.t.	Tc
GOD 3	GO 73i-1			Molar	–	B.t.	T3
Göttingen FMZ, Germany							
GOE 1	Obj.1181 F.Nr.6521/2	Early LBK	Betty Arndt	Radius	B.t.	–	–
GOE 3	Obj.1222 F.Nr.806			Radius	B.p.?	–	–
GOE 4	Obj.777 F.Nr.761			Radius	B.p.	–	–
Goyet Cave, Belgium							
BIP 1	2230-2	27.500 cal. BC	Mietje Gemonpre	Tibia	Bison sp.	Bison sp.	Bison sp.
BIP 2	2230-1			Tibia	Bison sp.	Bison sp.	Bison sp.

Grotte Champeau, France	Early-mid. Palaeol.	L. Chaix			
GCH 1	—		Metacarpus	B.p.	—
GCH 2	—		Metacarpus	B.p./Bison ?	—
Grotte de la Bouloite, France					
BOU 1	—		Radius	B.p.	—
BOU 2	—		Tibia	B.p.	—
Grotte du Pardon, France	3340–3150 BC cal.	Louis Chaix	Radius	B.p.	Ph
PAR 1	G90.K23:d44:22				
Halle, Germany	97 A SF48	Hans-Jürgen Döhle	Calcane.	B.t., B.p. ?	Pa
HAL 1					
Haloula, Syria	A402.02Q:4j Est A10	C. Edwards, J.-D. Vigne			
SYR 26	sample'2 (A235)			Bos sp.	—
Herpaly House, Hungary					
HER 3	—	A. Choyke		—	—
HER 4	—			—	—
Hilzingen, Germany					
HIL 1	Gr.389-26, Bef. n.v	Elisabeth Stephan	Metacarpus	B.p.	—
HIL 3	Gr.394 Bef.963		Humerus	B.p.	—
HIL 5	Gr.159 2129		Tooth	B.t.	—
Hocacesme, Turkey	6700–4000 BC	H. Hongo, M. Özdoğan			
HC 4	4-HC '93 14N 0-21			Bos sp.	—
HC 6	6-HC '93 15N 527			Bos sp.	—
HC 8	8-HC '99 43			Bos sp.	T

(Continued)

Archaeological site, Laboratory code	Archaeol. code	Date	Given by	Skeletal element	Species Morphol.	Genetic	Haplo-type
Hódmezővásárhely- Bodzaspart, Hung.		Early Neolithic, Körös	István Vörös	Ulna	–	–	–
HOB 2	5.5.13.11						
Hódmezővásárhely- Gorza, Hung.		Late Neolithic	István Vörös	–	Bos sp.	–	–
HOD 2	68.8.47			–	Bos sp.	B.t.	T3
HOD 4	68.8.68						
Igüe du Gral, France			L. Chaix	–	Bison	–	–
IGU 1	P47.dec34 917	13680 BP					
Isernia, Italy		730.000 BP	Peretto	Bison?	–	–	–
ISE 2	Q 104, Settore-1, US3coll						
Kfar Hahoresh, Israel		PPNB	L. Kolska-Horwitz	Humerus	B.p.	–	–
KH 2	West R59 Hat.0.82/4.59/0.21						
Lod NY, Israel		Ceramic Neolithic	L. Kolska-Horwitz	Radius	B.t.	–	–
LOD 1	#201 Hat.175–180						
Mala Triglavca, Slovenia		Neolithic/Late Neol.?	Mihael Budja	Mandibula	B.t.	B.t.	T3
LJU 1	–						
LJU 2	–	Mesolithic		Incisivi	–	Bison	–
LJU 3	–	Neolithic/Late Neol.?		Atlas	–	B.p.	Pb

Maral Tappeh, Iran			Chalcolithic	M. Mashkour, C. Edwards, J.-D. Vigne					
IRQ 02	A404				Scapula	B.p. ?	-		
Mareuil-les-Meaux, France			Late Neolithic 5000-4900 BC	Rose-Marie Arbogast	Metatarsus Mandibula Costae Femur	B.t. B.t. B.p. B.p. ?	-	T3	
MAR 2	72 / 1678								
MAR 8	37 / 1854								
MAR 9	111 / 1760								
MAR 10	28 / 1832								
Mezra Tel Eilat, Turkey			> 6000 BC	H.-P. Uerpmann		B.p.	-		
MEZ 1	21 E-8					B.t.	-		
MEZ 2	21 E-10					B.p./Buffalo	-		
MEZ 3	26-1				Phalanx	B.t.	-		
MEZ 4	21-1				Phalanx	B.t.	-		
Mitterfeking, Germany			Münchshöfener Culture		G. Roth Tibia	B.t.	-		
KOEL 3	2-2								
Neustadt (Schl.), Germany			4500-4100 cal. BC	S. Hartz, U. Schmölcke	Phalanx I	Bos sp.	B.p.	Ph	
NES 1	LA 156/02 N 100-101 E 118-119								
NES 2	LA 156/04 N 100-101 E 116-117				Scapula	B.t.?	B.p.	Pf	
Nieder-Mörlen, Germany			Flomborn	Sabine Schade-Lindig					
NMR 3	10/2. 2507B/26704 EY99/1				Humerus, dist.	B.t., B.p. ?	B.t.	T3f?	
NMR 19	4/2. 1162/25890 EY98/2				Tooth	B.t. ?	-		
NMR 22	4/2. 1162/25890 EY98/2				Tooth	B.t. ?	B.t.	T3	
NMR 24	7/1. 877/25191				Humerus, prox.	B.t. ?	B.t.	T3	(Continued)

Archaeological site, Laboratory code	Archaeol. code	Date	Given by	Skeletal element	Species Morphol.	Genetic	Haplo-type
Orlovez, Bulgaria		earliest Neol., Karanova	Hans-Peter Uerpmann				
KAR 1	9			-	B.t.	-	-
KAR 3	8,A1B			-	B.t., B.p. ?	-	-
Polgár-Csőszhalom, Hungary		Late Neolithic	István Vörös				
POL 1	60.9.669			-	-	-	-
POL 2	60.9.197			-	-	B.t.	Tb
POL 3	60.9.1316			-	-	-	-
POL 4	60.9.1409			-	-	B.t.	T3
POL 5	60.9.1879			-	-	B.t.	T3
Quenstedt, Germany		Bronze Age	Hans-Jürgen Döhle				
QUE 1	77:193			-	B.t.	B.t.	T3a
QUE 2	74:52			-	B.t.	B.t.	T3
QUE 3	77:200			-	B.t.	B.t.	Ta
Rosenhof, Germany			S. Hartz, U. Schmölcke				
ROS 1	Ros74 VI A 148i	4838 +/- 81 cal. BC		Metatarsus	B.t.	B.p.	Pd
Ruffey-sur-Seille, France		Mesolithic	R.-M. Arbogast				
RUF 1	3-16994	Sauveterrien ancient		Metatarsus	B.p.	-	-
RUF 2	95 Niv III 1-15316	Sauveterrien ancient			B.p.	-	-
RUF 3	1-17508	Sauveterrien ancient		Tibia/Radius	B.p.	-	-
RUF 4	8145	Sauveterrien moyen		Tibia?	B.p.	B.p.	Ph
RUF 5	1-18993	Sauveterrien moyen		Humerus	B.p.	-	-
RUF 6	11196	Sauveterrien moyen		Metacarpus	B.p.	-	-
RUF 7	1 Niv II	Mésolithique récent		Tibia	B.p.	-	-
RUF 8	4522 Niv. IV	Mésolithique récent		Radius	B.p.	-	-

Schwanfeld, Germany SWA 1	SF 762-14	Oldest LBK	H.-P. Uerpmann	Tooth	Bos sp.	–	–
Shams-ed-Din, Syria SED	Sed A4 aa	6000 BC Halaf	H.-P. Uerpmann	Phalanx 1	B.t.	–	–
Siegsdorf, Germany Sieg 1	Fig. 4 Table 9 in Ziegler 1994	47 000 BP	W. Rosendahl		Bison b.	Steppe bis.	
Svodin, Slovakia SVO 1	1159 SBSK 4103, 49	Lengyel, 3000 BC	Hans-Peter Uerpmann		B.t.	B.t.	T3
SVO 2	SVBA 0625 / 46				B.t.	B.t.	T3
SVO 3*	SVBA 0625 / 56				B.t.	B.p.	P
Szegvár-Tűzköves, Hungary SZE 1	72.1.260	Neolithic	István Vörös		–	B.t.	T3
SZE 2	72.1.174				–	B.p.	P
Tell Brak, Syrien TB 03**	TB94 A1077:2/HS	Chalcolith. BZ	K. Dobney/ C. Edwards		Bos sp.	B.t.	(T?)
TB 07**	TB95 A1136:2/HS3	4.-3. JT Phase IV, late 3rd. Mill.			Bos sp.	B.t.	T3
Tel Hreiz, Israel THE 2	30/93 27/38	PN	L. Kolska Horwitz	Radius	B.t.	–	–
Tall-i-Mushki, Iran IRQ 09	A419	8.-9. Jt. BC	C. Edwards, J.-D. Vigne, M. Mashkour	Tooth	–	–	–
Trebur, Germany TRE 1	LFD AD EV 1988:79 Grave 90	Middle Neolithic	Holger Göldner	Humerus	B.t.	B.t.	T3 (Continued)

Archaeological site, Laboratory code	Archaeol. code	Date	Given by	Skeletal element	Species Morphol.	Genetic	Haplo-type
TRE 2	LFD AD EV 1988:79 Grave 60			Tibia prox.	B.t.	-	-
TRE 3	LFD AD EV 1988:79 Gra. 113			-(calf)	B.t.	B.t.	T3
TRE 4	LFD AD EV 1988:79 Grave 63			Humerus	B.t.	B.t.	T3
Trosly-Breuil, France		Neolithic	Rose-Marie Arbogast				
TRO 2	TB 89 K XX /23 76			-	B.t.	-	-
TRO 3	TB 89 K XIX /9 20			-	?	-	-
TRO 4	TB 89 K XX /16 59			-	B.t.?	-	-
TRO 10	TB 90 MXI/8 1/4 SW (10)			Metacarpus	B.p.	-	-
TRO 11	TB 89 KXIX/5 (16)			Metacarpus	B.t.	-	-
TRO 12	TB 87 DVIII 21 91 (12)			Metacarpus	B.t.	-	-
TRO 13	TB 0 87 EV III 27 (13)			Metacarpus	B.t.	-	-
Viesenhäuser Hof, Germany		LBK	Elisabeth Stephan				
VIE 1	Bef.9 2111/329 Nr.1317	middle/younger LBK		Tibia	B.t.	-	-
VIE 2	Bef.2 2423/1752 Nr.588	late/middle LBK		Humerus	B.p.	-	-
VIE 4	Bef.4 2510/199 Nr.1301	middle/younger LBK		Radius	B.p.	-	-

VIE 13	Bef.1 2622/1606, Nr.332	late LBK	Humerus	B.p.	–
VIE 14	Bef.6 2205/891 Nr.2156	middle/younger LBK	Tooth	B.t.	–
VIE 18	Bef.2 3435/2221 Nr.1853	middle/younger LBK	Tibia	B.t.	–
VIE 24	Bef.6 2840/1362 Nr.5138	LBK	Metacarpus	B.t.	–
VIE 25	Bef.1 2201/944 Nr.5078	LBK	Metatarsus	B.t.	–
Wängels, Holstein, Deutschland					
WAN 1	LA 518/1998	3946+/-79 cal BC		B.t.	T3
WAN 2	LA 505, 04 97/8	ca. 6000 BC		B.t.?	T3d
Yilan, Türkei					
YIL	R1-E51		1 Phalanx	B.p.	–

U. Schmölcke,
S. Hartz

H.-P. Uerpman

Precautions during ancient DNA analyses

The laboratories in Mainz are dedicated to ancient DNA only and fulfil the highest international standard and criteria for DNA clean rooms. The pre- and post-PCR (polymerase chain reaction) areas are strictly separated in two different buildings. A one-way-system avoids carry-over contamination: persons are only allowed to enter the pre-PCR lab with freshly washed clothes but entry is not permitted if the person has already been to another lab or the office on the same day. In an extra room clothes are changed with special clean room overalls, shoe covers, gloves, facemasks and face shields. All items are irradiated with UV light before they enter the lab. The rooms and workbenches are regularly cleaned with soap and bleach, and UV irradiated overnight. The water used for cleaning is irradiated with a water-proof UV bulb for at least ten hours.

Sample preparation was performed as follows. First, the bones were irradiated with UV light. In order to remove contaminations, the surface of the bones was removed. Approximately 2 by 1 by 0.5 cm were cut out of the bone and additionally irradiated. All extraction and amplification reactions were accompanied by blank controls. For authentication of the sequences each sample was extracted independently at least two times, followed by one or two PCRs, respectively. Randomly chosen PCR products were cloned. The results were only accepted when all sequences were consistent. For two samples (see Table 1), bone preparation, extraction, PCR and sequencing were independently reproduced in the Smurfit Institute of Genetics, Trinity College Dublin.

Extraction, PCR, sequencing and cloning

The extraction was performed as described by Burger *et al.* (2004). The analysed fragment of interest is determined by the use of specific starter molecules (primers). Three different primer pairs for the mitochondrial HVR 1 were designed and none of them were found to amplify human DNA. The third primer system has two different lower primers that give a longer and a shorter product, in order to get a haplogroup determination even for samples where the DNA was highly fragmented.

The ancient DNA was amplified by PCR (polymerase chain reaction) technique. The success of the PCR was checked on a 2% agarose gel. Afterwards the DNA was purified, sequenced and subsequently analysed on 310 Genetic Analyzer (Applied Biosystems). Randomly chosen PCR products were additionally cloned in order to monitor possible background contaminations and postmortal sequence damage. Detailed protocols of all steps are described in Bollongino (2005).

RESULTS

Out of 161 samples, 65 (including seven bison samples for comparison) were reproducibly amplifiable. The success rate within European samples was 52.1%. Within the Near Eastern samples less than 10% were amplifiable, demonstrating the bad DNA preservation in hot climates.

Before trying an interpretation of the results of the ancient samples, it is necessary to have a look at extant cattle populations. Modern taurine cattle can be divided into five groups (T, T1, . . . , T4, as described in Troy *et al.* 2001), so called haplogroups (see Fig. 1a). A haplogroup comprises all sequences ('haplotypes') that can be derived from a specific ancestral sequence. The best way to detect an ancestral sequence is to draw a network (see Fig. 1b). A network represents all types of sequences as circles that are connected through branches. These branches show the positions at which the respective sequences differ from each other. A haplogroup often appears in a starburst pattern, showing the ancestral sequence in the centre. The different sequences within one haplogroup are called haplotypes.

A network of the ancient sequences is shown in Fig. 1b. Two major clusters can be identified, one comprising the ancient cattle sequences and the other cluster showing all ancient aurochs. These groups are separated by at

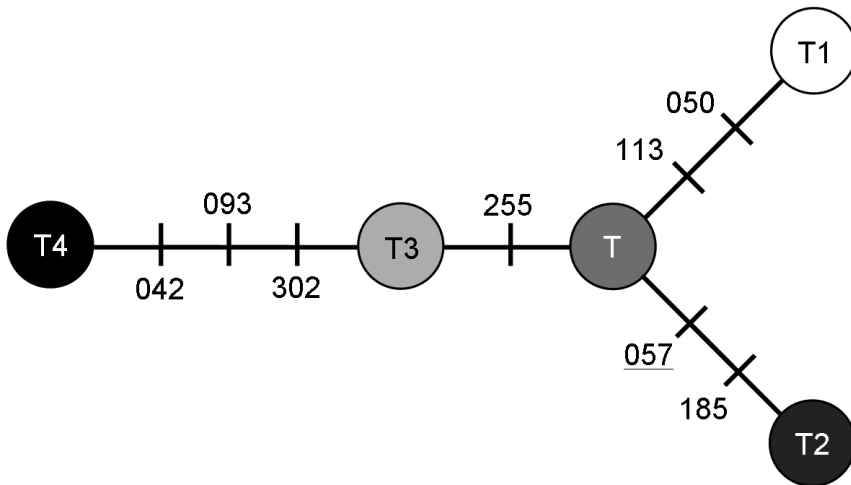


Figure 1a. Skeleton network showing the haplogroups of extant taurine cattle. The numbers indicate the positions of mutations (16.000+, the positions refer to the European consensus sequence with the GenBank accession no. NC_001567, Anderson *et al.* 1982) that define the respective haplogroup (for example haplogroups T3 and T can be distinguished by different bases at the position 16255). Haplogroup T4 can only be found in Eastern Asia, T1 is predominant in Africa. T2 is also present in Europe (but rarely) and the Near East, but could not be found within the ancient data set.

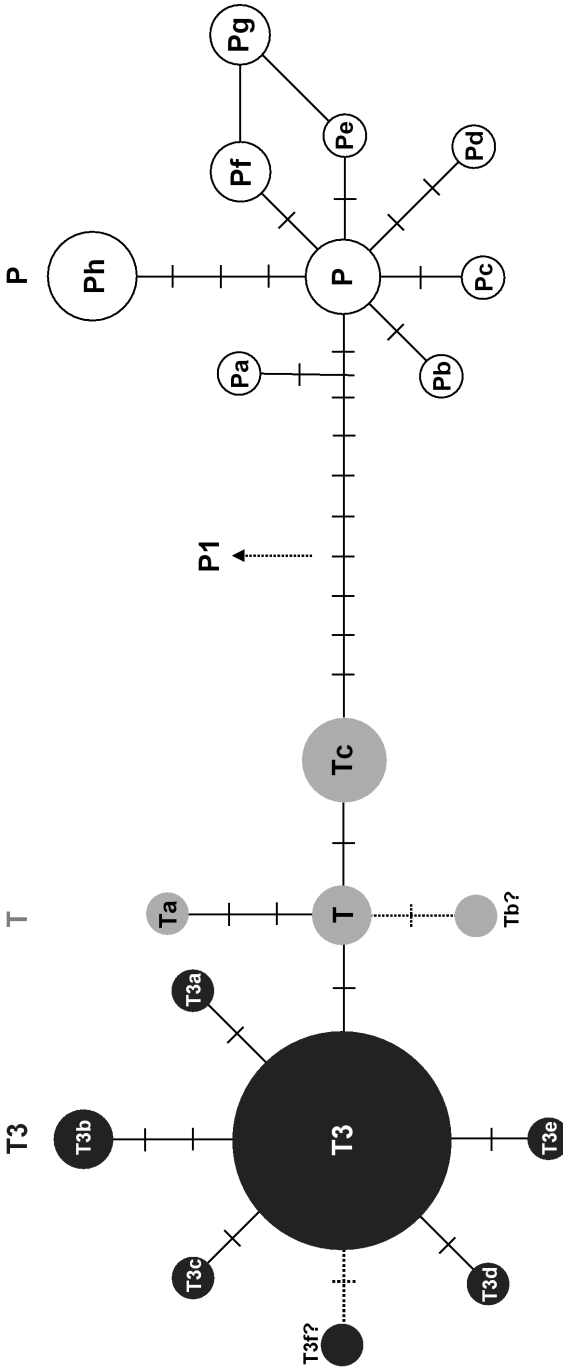


Figure 1b. Median Network of ancient sample sequences. Each circle represents a haplotype; the size is relative to the frequency of the haplotype. Each dash marks a mutation. The haplotypes show a star-like formation with the ancestral sequence in the centre. All haplotypes that descend from one ancestral sequence belong to the same haplogroup (T3 = black, T = grey, P = white, P1 = sample ELL4). Haplotypes with a question mark indicate samples that could not be amplified for all fragments, thus leaving some insecurity about possible further mutations. The network was drawn using the method described in Bandelt *et al.* (1995).

least nine mutational steps. The cattle sequences belong to the haplogroups T and T3. The majority of the cattle sequences belong to the central haplotype of T3. T3 is the most dominant haplogroup within modern European cattle whereas T is very rare. In the Near East both T and T3 are distributed.

The sequences of the ancient samples were compared to modern data from taurine and zebu cattle and European bison in a neighbour-joining tree (Fig. 2). Water buffalo is the outgroup, followed by wisent and zebu. The modern cattle data cluster together with the ancient cattle samples, whereas the ancient aurochs are the neighbour group of taurine cattle. None of the extant sequences belongs to the aurochs clade. One sample (EIL 4) has a very unusual sequence (haplotype P1) and neither belongs to the aurochs nor the cattle cluster. A comparison with the sequences in GenBank (internet database) revealed that it is a *Bos* sequence, but has no close similarity to any known breed.

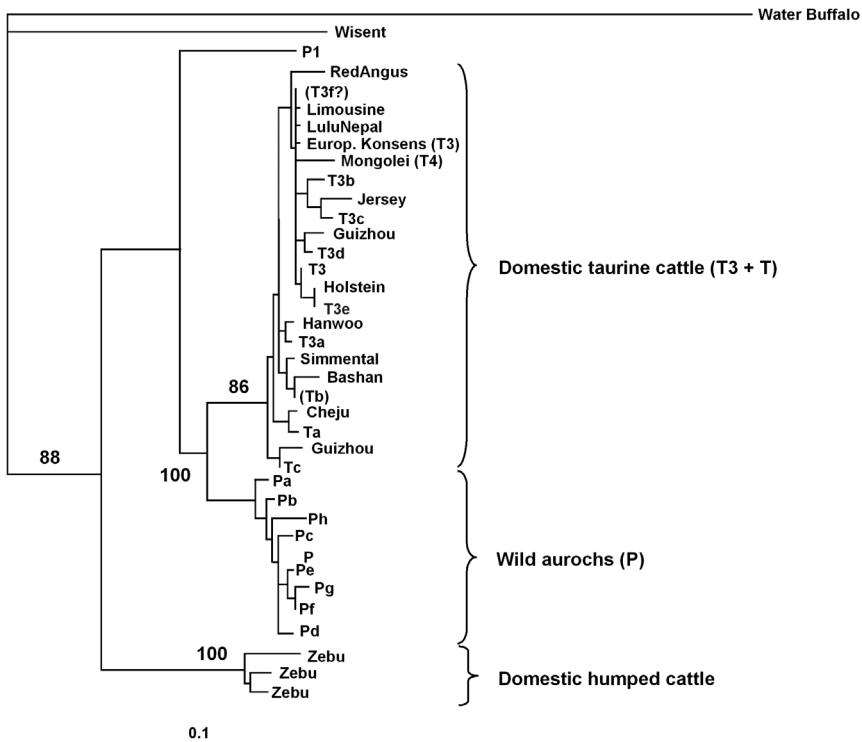


Figure 2. Neighbour-joining tree of ancient and modern sequences. The bootstrap values at the branches indicate how many of 100 calculated trees showed exactly this branch. The small letters represent single haplotypes within the respective haplogroup referring to the network in Fig. 1. P1 is the uncommon haplotype of the sample EIL4. The tree was calculated with PAUP (Swofford 2002).

DISCUSSION

Authentication

The sequences are regarded as authentic for the following reasons. Contaminations during the lab procedure can effectively be ruled out, as all extraction and PCR blank controls were blank. Cross contaminations did not occur as both aurochs and cattle samples were extracted and amplified contemporarily, and none of the aurochs samples ever showed a taurine sequence or vice versa. Many of the sequences are unique and the aurochs lineage is extinct, which means that it cannot be found within modern data and thus cannot stem from recent contaminations. The aurochs sequences are identical, or very similar, to those previously published by Bailey *et al.* (1996) and Troy *et al.* (2001). All results were extensively reproduced (see materials and methods) and two samples were independently reproduced in Dublin. Random cloning showed that no background contamination could be found. Post-mortem damages, such as deaminations (Gilbert *et al.* 2003a; 2003b; Hansen *et al.* 2001; Hofreiter *et al.* 2001), were ruled out by reproduction of sequences and use of UNG. For four samples (two cattle [SVO 1 and EIL 2] and two aurochs bones [SVO 3 and EIL 6]), an additional amplification of the cytochrome *b* locus was performed (Czerwinski 2003). In contrast to the d-loop, the cytochrome *b* is a coding gene and thus can be translated into the amino acid sequence. The translation showed that the amino acid sequence is correct so that reproducible post mortem sequence changes can be excluded (data not shown). Two variable positions could be revealed (positions 14873 and 15134) and both are silent mutations (that is, they do not affect the encoded amino acid), thus underlining the authenticity of the sequences. Additionally, the analysis of the results showed that all data make phylogenetic sense.

Genetic distinction of *Bos taurus* and *Bos primigenius*

A clear difference between *B. taurus* and *B. primigenius* is not necessarily expected because the aurochs is the ancestor of the domestic cattle. They share the same molecular background so that a strong genetic similarity would not be surprising. But our data speak for a rather distant relation between the two as both the neighbour-joining tree (Fig. 2) and the network (Fig. 1b) divided all data in two major groups. The large distance of nine mutations suggests a clear genetic difference between cattle and aurochs. We believe that one of the groups (P, see Fig. 1b) represents the aurochs for the following reasons. This group contains only sequences that belong to an

extinct lineage while the cattle haplogroups are identical to modern ones. Two samples (ETI 1, RUF 4) date to the Mesolithic, which is definitely prior to the first domestication and shows a typical aurochs haplotype. Our aurochs haplogroup is identical to those that have previously been published by Bailey *et al.* (1996) and Troy *et al.* (2001). Furthermore, the majority (90%) of the samples that were analysed by morphometric means supported the genetic classification of the sequences in aurochs and domestic cattle (see Table 1). The distinction between *B. taurus* and *B. primigenius* is also revealed by the cytochrome *b* results. Compared to the d-loop, this locus is very conservative and hardly shows any polymorphisms within one species. The two differences (for positions see above) between cattle and aurochs underline the genetic distance between the two groups.

The taxonomic status of the sample EIL 4 cannot be identified completely by the current data. The morphology of the bone is very robust and above the size variation of Neolithic cattle, and therefore the morphometric analysis clearly addresses this sample as an aurochs. It is possible that this individual represents a different population that might stem from another glacial refuge, maybe from a region in Asia, but aurochs sequences from this geographical part of the world are not known yet. The final evaluation of the EIL 4 sample has to be left for future research.

Differences in morphometric and genetic classification of *Bos taurus* and *Bos primigenius*

Within the samples that were morphometrically determined, the consistence with the genetic classification was 90%. Thus both methods confirm each other for the great majority of bones. The few differences can be explained by several possibilities. First, bones of a medium size are difficult to classify due to sexual dimorphism; that is, it is not possible to tell whether the bone comes from a female aurochs or a domestic bull. Secondly, the animal could be a hybrid. For example, if the mother was a domestic cow and the father an aurochs bull, the offspring may have had a rather aurochs-like phenotype, but the mitochondrial matriline would identify it as domestic cattle. In order to solve such a case, an additional analysis of a patrilinear marker is necessary. These loci can be found on the Y-chromosome in the nucleus, but is very difficult due to the very low copy number. Nevertheless, few Y-chromosomal sequences from ancient wild and domestic cattle could be amplified. Unfortunately the investigated locus (zinc finger gene) did not show any polymorphisms. The low variability does not allow us to distinguish patrilines of aurochs and domestic cattle (Bollongino 2005).

The origin of European cattle and their relation to the European aurochs

The results of this study do not support the theory of an indigenous origin of European domestic cattle. In the case of an independent secondary domestication, the mitochondrial sequences of *B. taurus* and *B. primigenius* should be almost identical. But even Early Neolithic cattle samples, like those from Eilsleben and Goddelau in Germany, are very distant from their contemporary aurochs sequences, and thus European aurochs cannot be the progenitors of domestic cattle.

So where do domestic cattle originate? A possible centre of origin, from the archaeological and archaeozoological context, is the Anatolian and Near Eastern region. There has also been some discussion, initiated by Bökönyi (1974), about a local domestication in Hungary. We analysed samples from two sites that were addressed as possible domestication centres (Polgár and Berettyószentmárton), plus two additional Hungarian sites (Szevár-Tüzköves and Albertfalva). But the cattle sequences from these sites (POL 2, POL 4, POL 5, ALB 1, ALB 3 and SZE 1) as well as the aurochs data (SZE 2, ALB 2 and ALB 4) show the same haplogroups as the respective Central European samples and, most importantly, show the same distance too. Therefore our data do not support the theory of an independent domestication in Hungary.

As Central Europe and the Balkans can be excluded as domestication centres, the Near East and Anatolia remain the most likely origins. And indeed the ancient samples from this region (TB 07, CH 11, AP6, HC 8) belong either to haplogroup T or T3, whereas the European aurochs haplogroup P can be found in neither ancient nor extant Near Eastern cattle.

Even if all cattle were imported into Europe, it is still possible that the European aurochs contributed to the domesticated population by subsequent interbreeding. Genetically, there are two ways of interbreeding: male and female introgression. Female aurochs might be caught as calves and added to the herds in order to compensate for loss due to disease or a harsh winter. But archaeological findings showed that an extensive trading system connected the settlements, and it might have been easier to get domesticated animals from neighbours, rather than taking the risk of introducing the uncontrollable behaviour of wild aurochs. Male introgression could have happened when cattle herds were driven to the forest for feeding and cows were (on purpose or unintentionally) not kept separated from wild bulls. Both ways would leave traces in the genome. Female introgression of wild aurochs cows would have left aurochs matrilineal lines in modern cattle populations. If female introgression occurred, it was a rare event and not a successful one, either. The question of male introgression cannot be answered with the current data as

no ancient aurochs patriline are known yet. Such data can only be obtained by analysis of nuclear loci, such as the Y-chromosome, which are, as already mentioned, unfortunately not informative so far.

The fact that European wild oxen and domestic cattle are so distant from each other suggests that aurochs populations in Europe are different from those in the Near East. It is completely unknown where the glacial refuges of the aurochs were, but it seems that the post-glacial aurochs repopulation of Europe did not start from Near Eastern regions.

SUMMARY

This study revealed ancient mitochondrial data from 40 domestic cattle and 17 aurochs samples (plus ancient bison for comparison), which date mainly to the Neolithic, but which also includes some of Mesolithic and Bronze Age date. A genetic distinction of *B. taurus* and *B. primigenius* within Europe could be shown. The large molecular distance between the two groups, even in the Early Neolithic, excludes an independent domestication of European cattle. All European domestic cattle haplogroups could be traced back to the Near East. A suggested secondary domestication centre in Hungary could not be supported. Furthermore, there are no genetic traces of interbreeding of imported cattle and European aurochs.

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