

**TITLE: Early history of European domestic cattle as revealed by ancient DNA.**

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### **Supplementary information**

Table S1. Table of archaeological samples studied, with associated codes, skeletal element used, and additional information supplied. Site details and dates are given above each set of specimens. Archaeological dates are based on the context of, and evidence from, each excavation. Phenotype versus mitotype and maximum mitochondrial region sequence lengths are included. Key: \* partial sequence generated previously by Edwards *et al.* (2004). Samples that have been analysed in both Mainz and Dublin are shown in bold type. Site references were supplied by the archaeologists involved.

Table S2. Primers used to assay mitochondrial haplotypic variation in the control region of ancient cattle surveyed. Primers labelled Bos were designed in Mainz, while the AN primers were designed in Dublin (Troy *et al.* 2001; Edwards *et al.* 2004). In the case of the primer sets named BosH and BosL, the numbers refer to the position of the 3' end. The reference for all primers is the reviewed Anderson Sequence (Anderson *et al.* 1982) with the accession number NC\_001567.

Table S3. Details of samples included in each haplotype named in Table 1, main paper. Samples in bold were replicated in Mainz and Dublin.

Figure S1/Table S4. Distribution of deaminations of all GC positions. The three primer systems refer to the first three primer pairs in table S2 (supplementary information). Hotspots

are marked with black columns and asterisks. The numbers on the x-axis refer to the sequence positions (GENBANK accession NC\_001567) given in the table below, where shaded boxes indicate hotspots.

Hansen *et al.* (2001) showed that post mortem damages mainly consist of transitions, and defined two groups: type 1 (A/T→G/C) and type 2 (G/C→A/T), which have different postmortem mutation rates (Hansen *et al.* 2001; Gilbert *et al.* 2003a/b). Hofreiter *et al.* (2001) showed that more than 90% of base alterations are due to deamination of cytosine residues, a type 2 transition.

We found only 2.6% of all substitutions to be of type 1 (after Hansen *et al.* 2001), and none of these were produced in more than one PCR. Therefore, we focused on G/C→A/T transitions (type 2 transitions) to test for possible deamination hotspots that could lead to incorrect sequence determination. Consequently, we refer to deaminations only as G/C→A/T transitions. Only direct sequences and sequences produced without prior use of UNG are taken into account (all data from Mainz). The hotspots were detected as follows: we performed a chi-square statistics with the null hypothesis,  $H_0$ , being that there are no deamination hotspots. In order to determine the expected deamination frequency for each primer system, the average deamination rate,  $R_d$ , was calculated as follows:  $R_d = D / S \times P$ , where  $D$  is the total number of deaminations,  $S$  is the total number of sequences, and  $P$  is the total number of GC positions. The observed deamination frequency,  $O_d$ , at a given GC position is:  $O_d = D_p / S$ , where  $D_p$  is the number of deaminations at a given GC position. Using chi-square statistics, the observed deamination frequency at a given position was then tested against the expected frequency for the respective primer system.

A total of 21,399 GC positions out of 476 sequences were analysed. The average substitution rate (all kinds of non-reproducible deviations from the consensus sequence) is

1.6% (n = 351 substitutions). Most of these substitutions are type 2 G/C→A/T transitions (n = 331, equal to 94.3%) and, therefore, the average G/C→A/T deamination rate is 1.5%. The rate of type 1 transitions (A/T→G/C) is only 0.04%. The type 2 transversion rate is 0.007%.

84.4% out of 141 GC positions were affected by deamination, while only 22 GC positions were affected by no deaminations. When UNG was added to the PCR, only 0.05% of the GC positions resulted in G/C→A/T transitions (2 G/C→A/T transitions in 28 sequences). The chi-square statistics showed that 8.3% of all GC positions are affected significantly more often by deamination and, therefore, considered as hotspots. Therefore, the null hypothesis,  $H_0$ , that there are no deamination hotspots, can be rejected ( $P \leq 0.05$ ) for ten of the GC positions (as noted in both the diagram and table).

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site/ code	lab code	archaeological code	sourced by	site reference(s)	skeletal element (n/a = information not available)	place analysed (M = Mainz; D = Dublin; B = replication in both labs)	maximum sequence length (bp)	species (Bp = <i>Bos primigenius</i> ; Bt = <i>Bos taurus</i> ; ? = unknown)	
								phenotype	mitotype
<u>Mezra Tel Eilat, Syria - c. &gt;6000 BC</u>			Hans-Peter Uerpmann	not published					
	MEZ2	21 E-10			n/a	M	no amplification	Bt	x
	MEZ4	21-1			phalange	M	no amplification	Bt	x
<u>Shams-ed-Din, Syria - c. 6000 BC (Halaf)</u>			Hans-Peter Uerpmann	Uerpmann 1982					
	SED	Sed A4 aa			phalange	M	no amplification	Bt	x
<u>Teli Brak, Syria - c. 4000-2000 BC (Chalcolithic-Bronze Age)</u>			Keith Dobney	Bowman & Ambers 1989; Oates & Oates 1994; Emberling & McDonald 2002; <a href="http://www-personal.umich.edu/~pictrm/brakbib.html">http://www-personal.umich.edu/~pictrm/brakbib.html</a>	<b>vertebra</b>	<b>B</b>	<b>252</b>	<b>Bt</b>	<b>Bt</b>
<u>Asagi Pinar, Turkey - c. 5250-5080 cal BC (Karano IV Neolithic)</u>			Hitomi Hongo/Mehmet Özdogan	Benecke 1998; Karul et al. 2003; Pazinger & Schwarzberg 2005; <a href="http://www.canew.org/">http://www.canew.org/</a>					
	AP6	25-AP '97 131 18-			n/a	M	199	Bt	Bt
	AP7	26-AP '99 9R 136.-			n/a	M	361	Bt	Bt
<u>Cayönü, Turkey - c. 7000 BC (PPNB)</u>			Hitomi Hongo/Mehmet Özdogan	Braidwood et al. 1974; Lawrence 1982; Özdogan 1997; Öksüz 2000; Hongo et al. 2002, 2004; Turk 2004					
	CO1	19 1991 30M 5-13 R			n/a	M	no amplification	?	x
	CO2	2814 1987 25L 2-39 Lr			n/a	M	no amplification	?	x
	CO7	1991 29M7-15 R2			n/a	M	no amplification	?	x
	CO14	2672 1991 EF 7-6 Lr1			n/a	M	unreproducible	?	x
<u>Fikirtepe, Turkey - c. 6200-5500 BC</u>			Hitomi Hongo/Mehmet Özdogan	Özdogan 1999					
	FT1	10-FT 700			n/a	M	no amplification	?	x
	FT5	14 FT 19/20			n/a	M	no amplification	?	x
<u>Hoca Cesme, Turkey - c. 6700-4000 BC</u>			Hitomi Hongo/Mehmet Özdogan	Özdogan 1998					
	HC4	4-HC '93 14N 0-21			n/a	M	no amplification	?	x
	HC6	6_HC '93 15N 527			n/a	M	no amplification	?	x
<u>Didi Gora, Georgia - c. 2000-1000 BC (middle Bronze/early Iron Age)</u>			Hans-Peter Uerpmann	<a href="http://www.uni-tuebingen.de/ufg/juengere_abteilung/projekte/georgien.htm">http://www.uni-tuebingen.de/ufg/juengere_abteilung/projekte/georgien.htm</a>					
	DID1	DG 85-3-46			radius	M	unreproducible	?	x
	DID2	DG 85-3-11			calcaneus	M	no amplification	?	x
	DID3	DG 85-3-10			calcaneus	M	112	?	Bt
<u>Orlovce, Bulgaria - earliest Neolithic (Karano)</u>			Hans-Peter Uerpmann	not published					
	KAR1	9			n/a	M	no amplification	Bt	x
	KAR3	8.A1B			n/a	M	no amplification	Bt/Bp?	x
<u>Albertfalva, Hungary - c. 2500 BC (Bronze Age Bell Beaker)</u>			Alice Choyke	Choyke 1993; Bartosiewicz & Choyke 2002					
	<b>ALB1</b>	<b>65 obj 777 (small)</b>			<b>n/a</b>	<b>B</b>	<b>361</b>	<b>?</b>	<b>Bt</b>
<u>Berettyószentmárton, Hungary - Neolithic</u>			István Vörös	Bartosiewicz & Choyke 2002					
	BER1	56.11.186			n/a	M	unreproducible	?	x
	BER2	56.11.426			n/a	M	no amplification	?	x
	BER5	56.11.553			n/a	M	no amplification	?	x
	BER6	56.11.979			n/a	M	361	?	Bt
	BER8	55.4.132			n/a	M	no amplification	?	x
<u>Hódmezővásárhely-Bodzaspart, Hungary - early Neolithic (Koros)</u>			István Vörös	Gink 1974; Meier-Arendt 1990					
	HOB2	5.5.13.11			ulna	M	unreproducible	?	x
<u>Hódmezővásárhely-Gorza, Hungary - late Neolithic</u>			István Vörös	Gink 1974; Meier-Arendt 1990					
	HOD2	68.8.47			n/a	M	no amplification	?	x
	HOD4	68.8.68			n/a	M	361	?	Bt
<u>Polgár-Csöcszhalom, Hungary - late Neolithic</u>			István Vörös	Raczky 2000; Raczky & Meier-Arendt 2002					
	POL1	60.9.669			n/a	M	no amplification	?	x
	POL2	60.9.197			n/a	M	361	?	Bt
	POL3	60.9.1316			n/a	M	no amplification	?	x
	POL4	60.9.1409			n/a	M	361	?	Bt
	POL5	60.9.1879			n/a	M	361	?	Bt
<u>Szegvár-Tűzköves, Hungary - Neolithic</u>			István Vörös	Meier-Arendt 1990					
	SZE1	72.1.260			n/a	M	361	?	Bt
<u>Mala Triglavca, Slovenia - late Mesolithic</u>			Mihael Budja	Leben 1988					
	LJU1	unknown			mandible	M	361	Bt	Bt
<u>Svodin, Slovakia - c. 3000 BC (Lenyel late Neolithic)</u>			Hans-Peter Uerpmann	Némejcová-Pavuková 1986, 1995					
	SVO1	1159 SBSK 4103.49			n/a	M	361	Bt	Bt
	SVO2	SVBA 0625 / 46			n/a	M	361	Bt	Bt
	SVO3	<b>SVBA 0625 / 56</b>			<b>n/a</b>	<b>B</b>	<b>361</b>	<b>Bt</b>	<b>Bp</b>
<u>Bad Abbach, Germany - c. 5300-5100 BC (Neolithic)</u>			Georg Roth	Rind 2004					
	KOEL2	23-1			humerus	M	no amplification	Bt	x
<u>Derenburg-Steinkuhlenberg, Germany - c. 3600 BC (Bernburg Neolithic)</u>			Hans-Jürgen Döhle	Döhle & Stahlofen 1985; Döhle 1997a					
	DER1	HK 87:183i			distal tibia	M	361	Bt	Bt
<u>Eilsleben, Germany - c. 5000 BC (LBK)</u>			Hans-Jürgen Döhle	Döhle 1994, 1997a, 1997b					
	<b>EIL1</b>	<b>HK 83:1040 I</b>			<b>metacarpal</b>	<b>B</b>	<b>361</b>	<b>Bt</b>	<b>Bt</b>
	EIL2	HK 88:354k			humerus	M	361	Bt	Bt
	EIL7	HK 85:142			n/a	M	361	Bt	Bt
	EIL8	HK 78:169			n/a	M	no amplification	?	x
	EIL9	HK 85:138 p			distal metacarpal	M	361	Bt	Bt
	EIL11	78:324			n/a	M	no amplification	?	x
	EIL12	HK 78:162			n/a	M	361	Bt	Bt
	EIL13	HK 88:487 g			distal tibia	M	361	Bt	Bt
<u>Goddelau, Germany - oldest LBK</u>			Hans-Peter Uerpmann	Uerpmann 2001; Bentley & Knipper 2005					
	GOD1	GO 73F-2 90			femur	M	361	?	Bt
	GOD2	GO 9-217			pelvis	M	361	?	Bt
	GOD3	GO 73i-1			molar	M	361	?	Bt
<u>Göttingen FMZ, Germany - early LBK</u>			Betty Arndt	Arndt 1997, 1998, 2000; Wolf 1998					
	GOE1	Obj.1181 F.Nr.6521/2			radius	M	no amplification	Bt	x
<u>Hilzingen, Germany - LBK</u>			Elisabeth Stephan	Fritsch 1998					
	HIL5	Gr.159 2129			tooth	M	no amplification	Bt	x
<u>Mittertecking, Germany - c. 4500-3900 BC (Münchshöfener culture)</u>			Georg Roth	Powrozniak & Rind 1997					
	KOEL3	2-2			tibia	M	no amplification	Bt	x
<u>Nieder-Mörlen, Germany - Flomborn (middle Neolithic)</u>			Sabine Schade-Lindig	Lindig & Schwitalla 1999					
	NMR3	10/2. 2507B/26704 EV99/1			distal humerus	M	361	Bt/Bp?	Bt
	NMR8	1/1. 67168/19168 EV97/21			phalange	M	no amplification	Bt/Bp?	x
	NMR19	4/2. 1162/25890 EV98/2			tooth	M	no amplification	Bt?	x
	NMR22	4/2. 1162/25890 EV98/2			tooth	M	361	Bt?	x
	NMR24	7/1. 877/25191			proximal humerus	M	361	Bt?	Bt
<u>Quenstedt, Germany - Bronze Age</u>			Hans-Jürgen Döhle	Döhle 1997b					
	QUE1	77:193			n/a	M	361	Bt	Bt
	QUE2	74:52			n/a	M	361	Bt	Bt
	QUE3	77:200			n/a	M	361	Bt	Bt
<u>Schwanfeld, Germany - oldest LBK</u>			Hans-Peter Uerpmann	Uerpmann 2001; Bentley & Knipper 2005					
	SWA1	SF 762-14			tooth	M	no amplification	?	x

<u>Trebur, Germany - middle Neolithic</u>		Holger Gökner	Spatz & von den Driesch 1996; Spatz 1999					
TRE1	LfD AD EV 1988:79 Grave 90			humerus	M	361	Bt	Bt
TRE2	LfD AD EV 1988:79 Grave 60			proximal tibia	M	unreproducible	Bt	x
TRE3	LfD AD EV 1988:79 Gra. 113			n/a	M	361	Bt	Bt
TRE4	LfD AD EV 1988:79 Grave 63			humerus	M	361	Bt	Bt
<u>Viesenhäuser Hof, Germany - LBK</u>		Elisabeth Stephan	Biel 1983; Kurz 1991, 1992, 1993, 1994; Krusholz 1997					
VIE1	Bef.9 2111/329 Nr.1317			tibia	M	unreproducible	Bt	x
VIE14	Bef.6 2205/891 Nr.2156			tooth	M	no amplification	Bt	x
VIE18	Bef.2 3435/2221 Nr.1853			tibia	M	no amplification	Bt	x
VIE24	Bef.6 2840/1362 Nr.5138			metacarpal	M	unreproducible	Bt	x
VIE25	Bef.1 2201/944 Nr.5078			metatarsal	M	no amplification	Bt	x
<u>Ardèche, France - c. 5500 cal BC (early Neolithic)</u>		Jean-Denis Vigne	Roudil & Saumade 1981; Roudil 1987					
<b>Ardèche 01* BA66 ADN; BO83-N12.C46 Z.250</b>				<b>tooth</b>	<b>B</b>	<b>252</b>	<b>Bt</b>	<b>Bt</b>
<u>Bercy, France - c. 4000 cal BC (middle Neolithic)</u>		Anne Tresset	Balasse et al. 1997; Bocherens et al. 1997; Tresset 2000					
Bercy 13*	QS. MXI 3-C21; MB 89000			right mandible	D	252	Bt	Bt
<u>Mareuil-les-Meaux, France - c. 5000-4900 BC (late Neolithic)</u>		Rose-Marie Arbogast	not published					
MAR8	37 / 1854			mandible	M	361	Bt	Bt
<u>Trosly-Breuil, France - Neolithic</u>		Rose-Marie Arbogast	Arbogast 1993					
TRO2	TB 89 K XX / 23 76			n/a	M	112	?	Bt
TRO4	TB 89 K XX / 16 59			n/a	M	unreproducible	?	x
TRO11	TB 89 KXIX/5 (16)			metacarpal	M	no amplification	Bt	x
TRO12	TB 87 DVIII 21 91 (12)			metacarpal	M	no amplification	Bt	x
TRO13	TB 0 87 EV III 27 (13)			metacarpal	M	no amplification	Bt	x
<u>Kilgreany, Ireland - 5190 +/- 80 bp (Neolithic)</u>		Nigel Monaghan	Woodman et al. 1997					
Bt	NMING: F21731 (KE1.1214)				D	217	Bt	Bt

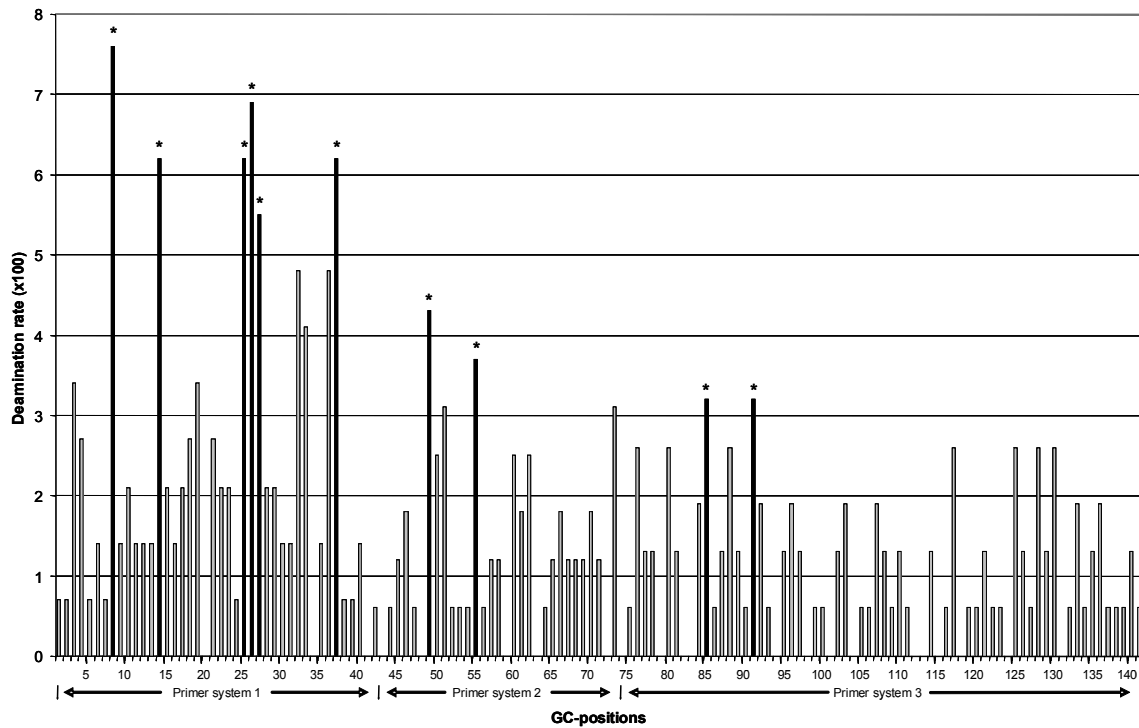
Supplementary information Table S1.

Primer (and position)	Sequence (5' to 3')	Product length (bp)
BosH15902	GCA CCC TAA CCA AAT ATT ACA AAC AC	173
BosL16024	GTC ATG TAC TTG CTT ATA TGC ATG GG	
BosH16040	TAT GCC CCA TGC ATA TAA GCA A	156
BosL16153	CGG CAT GGT AAT TAA GCT CGT G	
BosH16184	TAC CAT GCC GCG TGA AAC CA	175
BosL16313	TCC ATC GAG ATG TCT TAT TTA AGA GGA	
AN2F (16,022–16,041)	GCC CCA TGC ATA TAA GCA AG	157
AN1R (16,178–16,159)	AAT TAT ATG TAT TAT GTA CT	
AN1F (16,159–16,178)	AGT ACA TAA TAC ATA TAA TT	176
AN3R (16,334–16,314)	CGA GAT GTC TTA TTT AAG AGG	

Supplementary information Table S2.

sequence #	Neolithic samples	Bronze Age samples
1	BER6, DER1, EIL02, EIL07, EIL09, EIL12, EIL13, GOD1, GOD3, HOD4, LJU1, MAR08, NMR03, NMR22, NMR24, POL4, POL5, SVO1, SVO2, SZE1, TRE1, TRE3, TRE4	<b>ALB1</b> , QUE2
2	<b>Ardeche 01</b>	---
3	Bt	---
4	TRO2	DID3
5	---	QUE1
6	<b>EIL01</b>	---
7	---	<b>TB07</b>
8	AP6	---
9	Bercy 13	---
10	AP7, GOD2	---
11	POL2	---
12	---	QUE3
13	<b>SVO3</b>	---

Supplementary information Table S3.



Supplementary information Figure S1.

x-axis no.	1	2	3	4	5	6	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
mt position	15,903	905	908	909	913	918	921	922	923	924	928	930	932	934	935	937	939	943	949	950	953	954	955	
x-axis no.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
mt position	958	959	960	961	966	969	974	981	989	991	994	999	16,006	9	19	22	23	41	44	47	49	50	51	52
x-axis no.	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
mt position	57	58	60	63	70	74	82	84	85	86	89	93	96	102	104	110	114	117	121	127	139	140	144	145
x-axis no.	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
mt position	150	185	186	189	190	191	192	193	196	197	198	200	201	202	205	206	207	209	212	214	215	216	218	219
x-axis no.	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
mt position	220	221	222	223	224	225	231	232	233	235	236	237	238	239	241	242	243	247	248	252	260	261	263	264
x-axis no.	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141			
mt position	265	268	270	271	274	278	281	283	284	285	286	287	290	292	295	301	302	303	305	306	310			

Supplementary information Table S4.