Matrilines in Neolithic cattle from Orkney, Scotland reveals complex husbandry patterns of ancestry

Sheena Fraser[a1]*, Julia Elsner[b]*, W. Derek Hamilton[c], Kerry L. Sayle[c], Angela Schlumbaum[b], László Bartosiewicz[d]

[a] School of History, Classics and Archaeology, University of Edinburgh, William Robertson Wing, Teviot Place, Edinburgh, EH8 9AG; [b] Integrative Prehistory and Archaeological Science, Spalenring 145, 4055 Basel, Switzerland; [c]Scottish Universities Environmental Research Centre, University of Glasgow, Rankine Avenue, East Kilbride, G75 0QF, UK; [d] Osteoarchaeological Research Laboratory, Stockholm University, Lilla Frescativägen 7, 106 91 Stockholm, Sweden

[1] Present address; National Museums Scotland, National Museums Collection Centre, 242 West Granton Road, Granton, Edinburgh, EH5 IJA

*These authors contributed equally to this work.

Abstract

mtDNA, isotopic and archaeozoological analyses of cattle teeth and bones from the Late Neolithic site of Links of Noltland, Orkney, Scotland revealed these animals followed similar grazing regimes but displayed diverse genetic origins and included one cattle skull that carried an aurochs (wild cattle) genetic haplotype. Morphometric analyses indicate the presence of some cattle larger than published dimensions of Neolithic domestic cattle. Several explanations for these finding are possible but may be the evidence of a complex

pattern of domestic cattle introductions into Neolithic Orkney and interbreeding between domestic and wild cattle.

Keywords: Orkney, Late Neolithic, Links of Noltland, bovid mtDNA, stable isotope analysis, aurochs.

INTRODUCTION

The transition from foraging to producing economies (Neolithisation) triggered major changes in human prehistory. It started some 12,000–10,000 years ago in the Near East with the successive development of sedentism, plant cultivation, animal husbandry and the invention of pottery (Zeder, 2009). The Neolithic lifestyle spread into Europe via two main routes, along the Mediterranean coast and the Danube (Lichter, 2005). The first farmers entered the British Isles around 4,000 BC (Rowley-Conwy, 2011, Whittle, et al., 2011) and arrived in the northern islands of Orkney by approximately 3,500 BC (Ashmore, 2009). The "Neolithic package" included domestic taurine cattle (*Bos taurus* L., 1758) that descended from the Near Eastern variety of aurochs (*Bos primigenius* Bojanus, 1827) (e.g. Bollongino, et al., 2012, Edwards, et al., 2007).

Ancient DNA (aDNA) studies widely apply the analysis of the maternally propagated non-recombining mitochondrial DNA (mtDNA) which is abundant in cells and highly variable, particularly within the displacement loop (d-loop). Modern and ancient cattle mtDNA diversity has been intensively studied (e.g. Achilli, et al., 2009, Beja-Pereira, et al., 2006, e.g. Edwards, et al., 2007, Lenstra, et al., 2014, Olivieri, et al., 2015, Scheu et al., 2015, Troy, et al., 2001), and led to the identification of seven major haplogroups (hg) in wild and domestic cattle. Macro-hg T dominates in taurine and macro-hg I in indicine cattle (*B. indicus*, zebu).

Hgs P, Q, and R are very rare in modern cattle; and both hg E (Edwards, et al., 2007) and C (Zhang, et al., 2013) apparently did not survive in the domestic gene pool. It seems reasonable to expect that local wild individuals were sporadically incorporated into the domestic stock during their spread westward. These events, however, are difficult to detect morphologically and appear to be rare in central and northern Europe, and so far few distinctive traces within the matrilineages were detected (Edwards, et al., 2007, Schibler, et al., 2014). On the other hand, some hg Q and R in modern Italian and Iberian cattle breeds may indicate occasional adoption of female aurochs into the domestic stock in southern Europe (Lopez-Oceja, et al., 2015). Moreover Park, et al. (2015) found an increasing proportion of local European aurochs progeny in North-western Europe, particularly the British Isles. In Europe, over time, domestic cattle living in agro-pastoral relationships with humans out-competed their wild counterparts, which were also extensively hunted (Budiansky, 1992). British aurochs became extinct in the Bronze Age (Kitchener, et al., 2004, Yalden, 1999). In continental Europe the last known aurochs died in AD 1627 in the Jaktorów Forest, Poland (Kyselý, 2008).

Aurochs reached withers heights between 140–190 cm (Degerbøl and Fredskild, 1970); a pronounced sexual dimorphism prevailed. Geographical location appears to have influenced size, with early Holocene central European specimens being larger than those from Scandinavia and the rest of northern Europe (Lasota-Moskalewska and Kobrýn, 1990), which were, however, larger than southern and eastern European individuals (Bökönyi and Bartosiewicz, 1987, Wright and Viner-Daniels, 2014). A general size reduction in European aurochs was noted between the Pleistocene and Holocene but no evidence shows that aurochs further decreased in size between the Mesolithic and Bronze Age (Bartosiewicz, 1999, Chaix and Arbogast, 1999, Degerbøl and Fredskild, 1970, Grigson, 1978, 1969, Lasota-Moskalewska and Kobrýn, 1990).

Aurochs were larger than domestic cattle, but sizes between female *B. primigenius* and male *B. taurus* overlapped. There are examples indicating that the distinction cannot be reliably based on phenotypic stature alone, but in some cases it is possible to unambiguously attribute smaller cattle bones to aurochs since they predated the arrival of domesticates into the respective regions (Saville, et al., 2012). The situation is difficult from the Neolithic onwards, when domestic cattle and wild aurochs co-existed. In this study, we have selected sixteen Late Neolithic cattle bone and tooth samples from Links of Noltland, Orkney) for mitochondrial haplotyping and molecular sex identification to determine whether they belonged to *B. primigenius* or *B. taurus*. Samples were also investigated using morphometric methods and stable isotope analyses.

The Orkney site

Cattle mtDNA samples were obtained from Neolithic deposits from Links of Noltland (LON) located in Westray (HY 428 493), excavated between 2006 and 2015 by EASE Archaeology on behalf of Historic Environment Scotland (Figure 1).

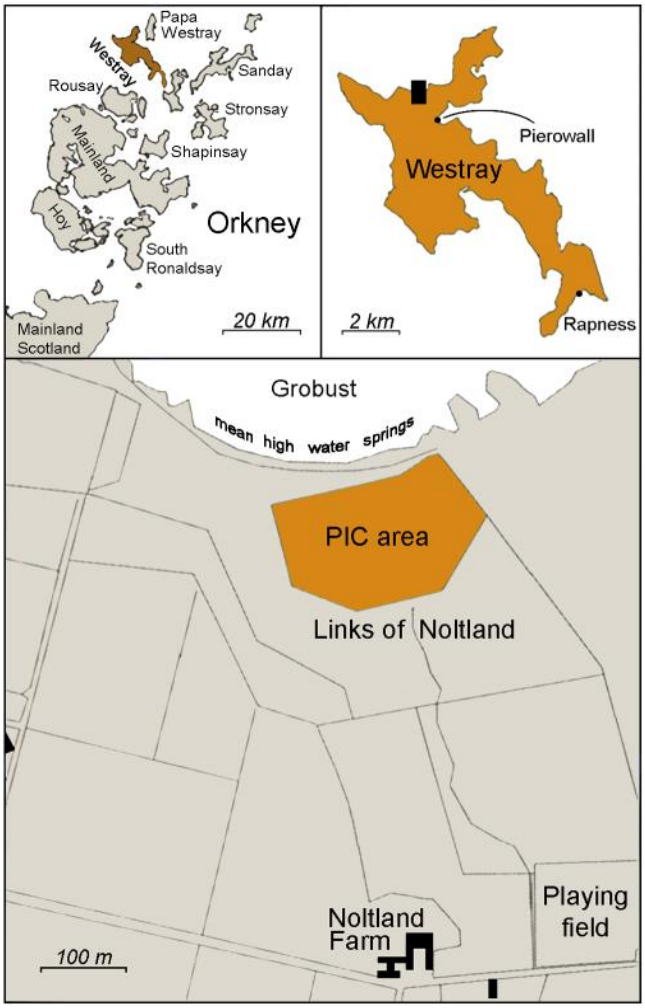


Figure 1. Position of Westray among the major isles of Orkney and location of the Links of Noltland excavated area (PIC) on Westray (Moore and Wilson, 2011)**.**

Westray is the most north-westerly island of Orkney, an archipelago of approximately 70 islands and islets with a total land area of 970 km^2 (Berry, 2000, Davidson and Henshall, 1989). Orkney (58.41–59.24^{o}N), located off the north-east coast of Scotland, is separated from the mainland by the Pentland Firth, a high-energy channel which links the Atlantic Ocean and North Sea (Bates, et al., 2013, Sturt, 2005). LON, a Scheduled Ancient Monument, covers an area of 3.5 ha. Excavation has uncovered an enclosed complex of Neolithic stone structures, as well as individual stone structures from the Neolithic and Middle Bronze Age, a cemetery, paths, field boundaries, cultivated soils and middens (Moore and Wilson, 2011). Mammal remains from this site were dominated by cattle (*Bos* sp.) and

sheep (*Ovis aries* L. 1758) with red deer (*Cervus elaphus* L. 1758) also present; a species no longer resident in Orkney.

For this study, five cattle samples were collected from a deposit of 28 cattle and two sheep skulls lodged within the inner and outer foundation courses of LON Structure 9 (Figure 2). This was a sub-rectangular or ovoid building, measuring 8 m by 8.8 m, located north of the main settlement. Deposition may have been undertaken by the LON community to confer some form of energy, spirit, defence or memory to this building, although the skulls would have been invisible after installation (Cauvin, 2000, Richards, 2013, Russell, 2012). Eleven further samples stem from midden deposits at the site (Table 1)

Figure 2. East Foundation, Structure 9, Skulls F6716, F6700, F4917 © G. Wilson

Table 1: Details of analyses undertaken with Links of Noltland (LON), Westray, Orkney cattle samples. Information in parenthesis could not be validated, ND = not determinable.

DNA laboratory code	GenBank accession for mt ht sequence	Molecular sex	LON context	Find number	Skeletal element	Archaeozoological analysis	SUERC code for samples used for ^{14}C dating and stable isotope analyses
ORK1	KU255585	ND	9123	F4257	Maxillary P^3 tooth	yes	51170
ORK2	KU255586	Male	9116	F4917	Mandibular M_3 tooth		51171
ORK3	KU255587	(Male)	9123	F6693	Maxillary M^1 tooth	Yes	51172
ORK4	KU255588	ND	9116	F4462	Maxillary P^2 tooth	Yes	51173
ORK5	KU255589	(Male)	9116	F4460	Maxillary M^2 tooth	Yes	51174
ORK6	KU255590	Female	9116	F4459	Maxillary P^3 tooth	yes	51175
ORK7	KU255591	Female	9129	F8442	Mandibular M_1 tooth		51176
ORK8	KU255592	Female	9021		Maxillary M^3 tooth		51182
ORK9	KU255593	Female	9021		Radius		51180
ORK10	KU255594	Female	9031		Radius		51181
ORK11	ND	ND	9017		Proximal phalanx		ND
ORK12	KU255595	(Male)	9028	Ass F4462	Metatarsus		51183
ORK13	ND	ND	9116		Humerus		51184
ORK14	KU255596	Male	9166		Proximal phalanx		51185
ORK15	KU255597	Female	9681	17528	Metatarsus		51186
ORK16	ND	ND	9681	17206	Metatarsus		ND

METHODS

Ancient DNA analyses and authenticity

DNA extraction followed the User Developed Protocol: "Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit" (Qiagen, Basel, Switzerland) for less than 100 mg, in double reactions for each sample. One mock control was performed per eight samples. All extracts were washed twice with water (molecular biology grade, Eppendorf, Allschwil, Switzerland) using 30 kD filter units (Amicon/Millipore, Zug, Switzerland). The final eluate was 200 µl.

Three targets of the mt d-loop covering nucleotide positions 15,903-16,023, 16,041-16,152, and 16,185-16,312 (*Bos taurus* reference sequence (BRS) V00654, Anderson, et al., 1982) were PCR amplified in 25 µl volumes containing 1.5 U AmpliTaq Gold, 1x GeneAmp 10x PCR Gold Buffer (150 mM Tris-HCl, 500 mM KCl, pH 8.0) and 2 mM $MgCl_2$ (all Applied Biosystems, Hombrechtikon, Switzerland); 0.4 mM dNTP Mix (Promega, Dübendorf, Switzerland); 0.2 µM of each primer; 20 µg/µl BSA (bovine serum albumin, Roche, Basel, Switzerland), and 3-9 µl template DNA on a Mastercycler ProS (Eppendorf, Allschwil, Switzerland). The cycling conditions were: 12 min initial denaturation, followed by 50 cycles of denaturation at 95 °C for 40 sec, annealing at 52-58 °C for 30 sec, and extension 72 °C for 30 sec, with a final extension of 60 sec at 72 °C. Non-template controls were performed alongside all amplifications. Additionally, molecular sex determination was attempted using sex-specific loci on the zinc-finger structures in the X and Y chromosomes (zfx and zfy). PCR conditions were as described above except that cycle number was increased to 70. Primer sequences for both mtDNA and zfx/zfy amplifications are given in Table S1. Successful PCR amplification was monitored on a 2% agarose gel, cut from the gel and purified with MinElute Gel Extraction Kit (Qiagen, Basel, Switzerland). PCR products were

premixed with elongated sequencing primers (Binladen, et al., 2007) and directly Sanger sequenced by Microsynth (Balgach, Switzerland).

To ensure authenticity of results, established standards in aDNA research at the Integrative Prehistory and Archaeological Science (IPAS) were adhered to (e. g. Elsner, et al., 2014, Schlumbaum, et al., 2010). This includes dedicated, physically separated laboratories for ancient DNA work (pre-PCR), and regular cleaning and UV radiation of surfaces, tools and consumables. No modern animal DNA was analysed in the post-PCR laboratory. Each target was validated on the basis of two independent extractions and at least two PCR products.

Genetic data analysis

Sequences were edited and aligned by eye using BioEdit (Hall, 1999). A consensus sequence was built following majority rule. In ambiguous cases the endogenous sequence was determined with a parsimonious approach, i.e., when a thymine would represent previously unknown haplotypes, we assumed the base was rather a deaminated cytosine as a result of *post mortem* damage than endogenous thymine. Considering the zfx/zfy loci, this can be neglected.

For comparison sequences were aligned with a total of 396 published Neolithic to Bronze Age *B. taurus* (Scheu, et al., 2015 and ref. therein) and *B. primigenius* sequences (Bollongino, et al., 2008, Edwards, et al., 2007, Scheu, et al., 2012). Samples were grouped into geographic bins which were, when appropriate, chronologically subdivided (Table S2). The alignment was pruned to BRS nucleotide positions 15,931-16,025; 19,051-16,152 and 16,185-16,312 to exclude missing data (threshold 2 %). Nucleotide and haplotype diversity as well as haplotype frequency were computed with Arlequin 3.5 (Excoffier and Lischer, 2010). To reject a statistical bias in the analyses introduced by uneven sample sizes in the respective geographic and/or time bins, directly compared bins were randomized (10k permutations with

replacement) using nucleotide and haplotype diversity estimated with the packages *pegas* (Paradis, 2010) and *seqinR* (Charif and Lobry, 2007) implemented in R (R Development Core Team, 2014) using the option pairwise deletion of missing data. Based on relative haplotype frequencies, Principal Component Analysis (PCA) was computed with PAST (Hammer, et al., 2001) using those geographical and time bins unbiased (in relation to the LON bin) by sample size. A Median Joining Network (MJN, Bandelt, et al., 1999) for the mtDNA sequences was constructed using the software Network (fluxus-engineering.com).

^{14}C-dating and stable isotopes

Radiocarbon (^{14}C) dating and stable isotope analyses (δ^{13}C, δ^{15}N and δ^{34}S) for cattle bone and teeth were determined by the Scottish Universities Environmental Research Centre (SUERC). Stable isotope analyses, particularly δ^{13}C and δ^{15}N, of preserved bone collagen have been extensively employed in palaeodietary reconstruction (DeNiro and Epstein, 1981, 1978) and can be used to identify whether the consumer was a herbivore, carnivore, or omnivore, as well as differentiate between terrestrial, freshwater, and marine-based diets (Schoeninger, 2014). In very recent years, analysis of δ^{34}S in bone collagen has proved to be extremely useful for distinguishing between the amounts of marine, freshwater, and/or terrestrial protein in a consumer's diet (Bocherens, et al., 2016, Privat, et al., 2007, Sayle, et al., 2014).

A modified version of the Longin method (Longin, 1971) was used to extract the collagen component from all bone and teeth samples. Sample surfaces were initially cleaned using a Dremel^{TM} multi-tool before they are lightly crushed into smaller fragments and immersed in 1 M HCl for approximately 24 hours to effect demineralisation. The acid was then decanted and samples were rinsed with ultra-pure water to remove any remaining dissociated carbonates, acid soluble contaminants and solubilised bioapatite. The gelatinous-like material was heated gently to approximately 80 °C in ultra-pure water to denature and solubilise the

collagen. After cooling, the denatured collagen solution was filtered, reduced to approximately 5 ml and freeze-dried. Approximately 15 mg of collagen was combusted in an evacuated sealed tube with copper oxide and silver foil at 850 ºC overnight. CO_2 produced was then isolated, cryogenically purified, and converted to graphite by reduction over zinc and iron. ^{14}C measurements were undertaken on either a National Electrostatics Corporation (NEC) 5MV tandem accelerator mass spectrometer or a 250 kV single-stage accelerator mass spectrometer (SSAMS) (Dunbar, et al., 2016). Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) were analysed using a Thermo Scientific Delta V Advantage continuous-flow isotope ratio mass spectrometer (CF-IRMS) coupled via a Thermo Scientific ConfloIV to a Costech ECS 4010 elemental analyser (EA) fitted with a pneumatic auto sampler. Bone collagen samples were weighed into tin capsules (~600 μg for $\delta^{13}C$ and $\delta^{15}N$, ~10 mg for $\delta^{34}S$) and measured as described by Sayle, et al. (2013).

Archaeozoological analysis

Cattle bones and teeth were measured using dial callipers following von den Driesch (1976). Measurements were to the nearest 0.1 mm, apart from *in situ* measurements which were to the nearest 1 mm. Buccal molar crown heights were measured from the boundary of the crown/root margin to the peak of the anterior lobe. Individual maxillary molar tooth circumferences, considered age-independent, were measured using cotton thread, following (Davis and Payne, 1993).

RESULTS

Ancient DNA analyses

Thirteen out of 16 samples yielded reproducible results for mtDNA haplotyping, and eight for molecular sexing (Table 1). While it was possible to verify PCR products for the zfx locus from all positive samples, for only one (ORK2) two independent products for the zfy locus

were obtained; four other individuals yielded only one zfy product which could not be validated despite nine to ten PCR attempts. Thus we could identify seven cows, one definite male and four probably male individuals. Males were present both in the deposit in structure 9 and in the midden.

Twelve samples belonged to maternal hg T3, associated with domestic cattle (*B. taurus*) in Europe. Beside six individuals belonging to the main European ht T3 (T3_1), two further T3 matrilineages were present in LON cattle: five cattle are T3_2 16074C and 16250 G, one cattle is T3_3 16122C and 16247T (Figure 3, Table S3). These hts are so far uniquely found at LON. One sample, a maxillary third premolar (P^3) from cattle skull ORK6, belonged to the most common European aurochs hg P, differing in 14 positions from the BRS and in positions 16'019, 16'141, and 16'301 from one *B. primigenius* reference sequence NC_013996 (figure 2, Table S3). Nucleotide (0.004) and haplotype (0.62) diversity of the T3 individuals are relatively high considering the remoteness of Westray compared to Neolithic cattle from the Near East and mainland Europe (Scheu, et al., 2015).

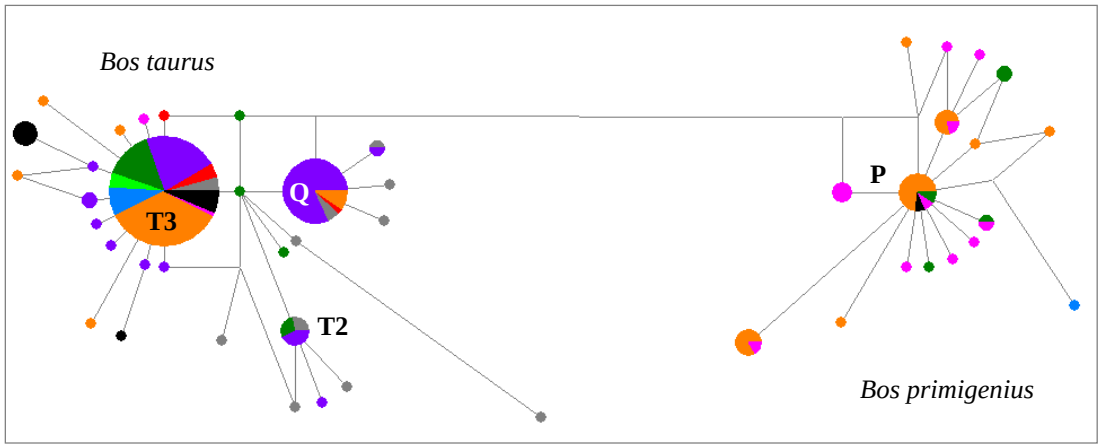


Figure 3: Median Joining Network of 409 archaeological *Bos* Genbank entries (Anderung, et al., 2005, Bollongino, et al., 2012, Bollongino, et al., 2006, Bollongino, et al., 2008, Edwards, et al., 2007, Scheu, et al., 2015, 2012) based on BRS nucleotide positions 15,931-16,025; 16,051-16,152 and 16,185-16,312. Circle size corresponds to the number of individuals. Iran, Syria – grey; Western Anatolia – red; Southeastern Europe – violet; Southeastern Central Europe – dark green; Italy – light green; Southern France – blue; Central Northwestern Europe – orange; mainland UK – pink. The LON individuals are in black.

Based on the randomization test relative haplotype frequencies of LON T3 samples were compared to Neolithic cattle from the Near East (Iran), Southeastern, Southern, Central, and Northern Europe (Figure 4, Tables S4, S5). Component 1 of the PCA graph explains 79 % variance and derives from the distribution of the most common haplotype within haplogroup T3. The matrilineages unique to LON influence component 2 (15 %). This combination sets the LON variability equally apart from the other Neolithic domestic cattle.

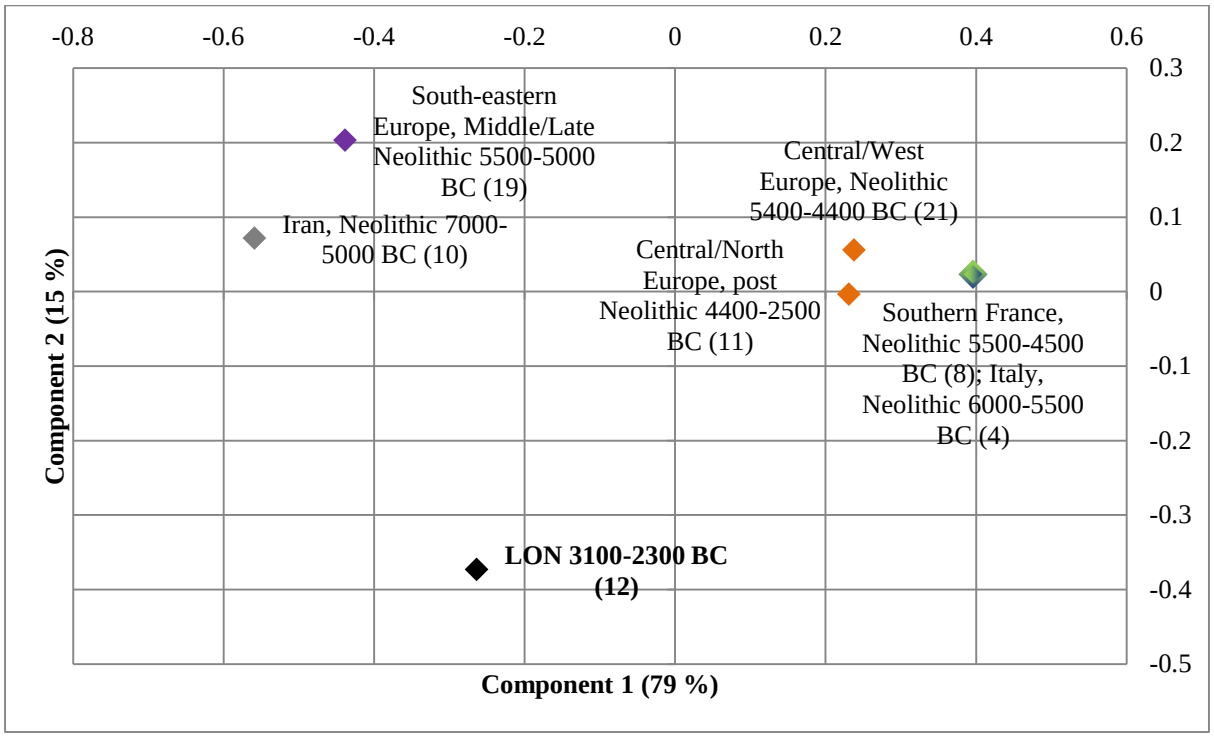


Figure 4: PCA graph based on relative haplotype frequencies of haplogroup T individuals. Component 1 and 2 explain 94 % of the variation. The graph contains geographical and time bins which allow an unbiased comparison with the LON samples.

Stable isotopes and ^{14}C-dating

All samples passed the quality criteria as set out by Ambrose (1990) and had C:N atomic ratios that fell within the range of 2.9 to 3.6, indicating good collagen preservation (DeNiro, 1985). With the exception of two samples (ORK14 and ORK15, italicized in Table S6), all passed the quality criteria for measuring sulfur isotopes in mammalian archaeological collagen as set out by Nehlich and Richards (2009), and displayed atomic C:S ratios within

600 ± 300, atomic N:S ratios within 200 ± 100 and contained between 0.15 and 0.35% sulfur. However, the isotopic results for ORK14 and ORK15 have been included within the discussion, as they pass the other standards as mentioned above.

Radiocarbon dating confirmed all tested samples were from the Scottish Late Neolithic (second half of the third millennium BC, Table S7). $\delta^{13}C$ values ranged from −21.7‰ to −20.4‰ (mean: −21.3 ± 0.3‰) and $\delta^{15}N$ values from +5.0‰ to +7.4‰ (mean: +6.1 ± 0.9‰) (Table S6) and were within expectation for animals consuming C_3 plants in Neolithic to Iron Age sites in Orkney and the Western Isles (Jones and Mulville, 2016). $\delta^{34}S$ values ranged from +16.8‰ to +20.8‰ (mean: +18.7 ± 1.1‰) and are typical for animals that have consumed vegetation that has been grown on coastal sites affected by sea-spray (Wadleigh, et al., 1994). The relationship between $\delta^{34}S$ and $\delta^{15}N$ showed a positive linear trend ($R^2 = 0.62$, Figure 5). While the elevated $\delta^{34}S$ values could be indicative of consumption of either seaweed or grasses affected by seaspray, the concurrent rise in $\delta^{15}N$ is suggestive of pasture fertilisation through manuring (Bogaard, et al., 2007). If marine vegetation was being actively eaten (e.g. seaweed on the shore) a similar positive linear relationship would be expected between $\delta^{13}C$ and $\delta^{34}S$. That there is no discernible pattern between these two stable isotopes suggests that Neolithic farmers in Orkney were using seaweed to fertilise pastures, thus only enriching $\delta^{34}S$ and $\delta^{15}N$, with $\delta^{13}C$ values reflecting expected values from atmospheric CO_2, and not the marine reservoir.

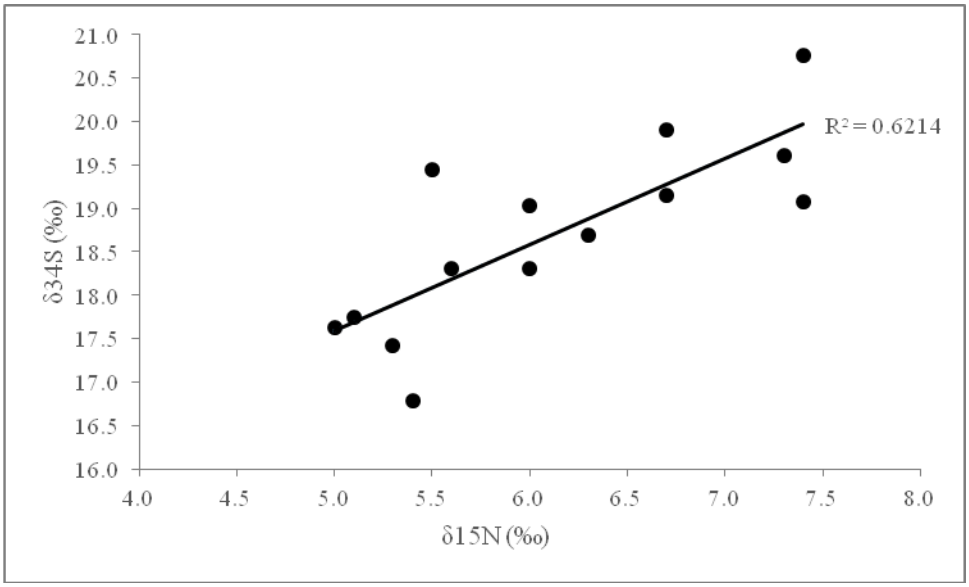


Figure 5: Plot of $\delta^{34}S$ versus $\delta^{15}N$ for bone and teeth from all 14 cattle that underwent radiocarbon dating and stable isotope analysis.

Archaeozoology

Cattle skull ORK6 (hg P) had the longest maxillary toothrow (P^2-M^3) compared with the other four hg T3 cattle skulls (Table S8). It was tabulated with published Danish measurements from female aurochs and Neolithic domestic cattle (Degerbøl and Fredskild, 1970) and Danish, Swedish and British female aurochs and female British domestic cattle breeds (museum collections) (Grigson, 1978, 1974). This toothrow was found to be longer than averages of female domestic cattle from Denmark and Britain, but below the minimum of female aurochs (Figure 6).

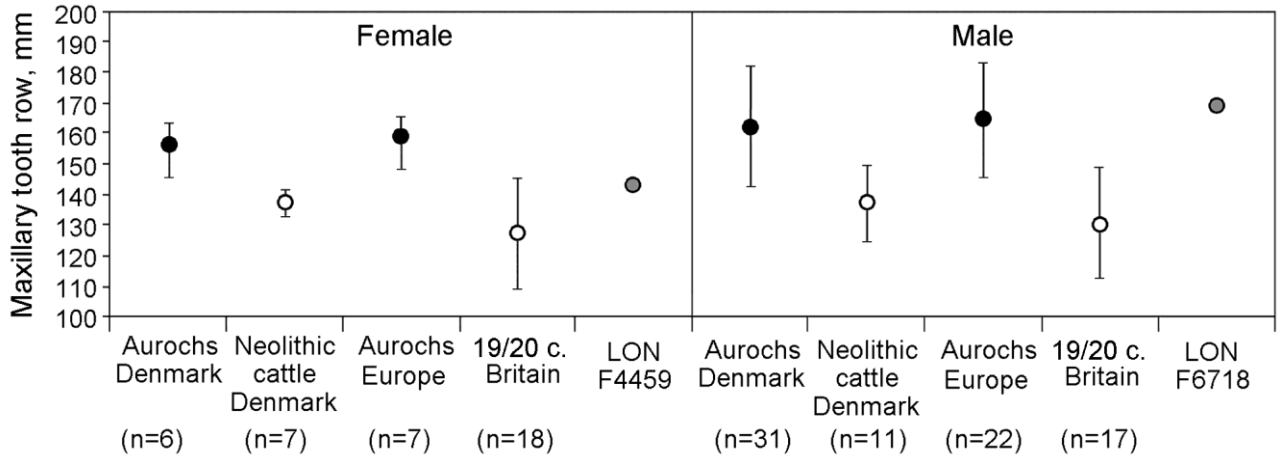


Figure 6: Lengths of the maxillary toothrow in cattle skulls F4459 (ORK6) and F6718 from LON in comparison with the mean, maximum and minimum values for female (left) and male (right) aurochs and Neolithic domestic cattle (Degerbøl and Fredskild, 1970, Grigson, 1978, 1974)

Individual maxillary molar measurements for cattle skulls tested for mtDNA were also examined (table S9). Based on tooth eruption four skulls were adults whereas ORK5 was a sub-adult (Simonds, 1854). Degerbøl observed a small overlap between molar tooth size in large domestic bulls and small aurochs but with breadth of molars being smaller in domestic cattle (Degerbøl and Fredskild, 1970). Cattle skull ORK6 had the highest ratio of occlusal breadth to length for all its molar teeth, the morphometric feature potentially associated with aurochs. These proportions could have resulted from a greater degree of tooth wear progressing with age, but the greater breadth to length ratio was not observed in cattle skull ORK1 (hg T3) which had similar molar crown heights.

In addition, all maxillary 3rd molar teeth (M^3) from LON skulls tested for mtDNA were within the range of basal circumferences listed for domestic cattle at the Late Bronze Age barrow 1 at Irthingborough and below the 116 mm measurement for an aurochs (Davis and Payne, 1993). For maxillary M^2, only ORK 6`s basal tooth circumference was greater than those listed for domestic cattle, but considerably smaller than that of aurochs and maxillary M^1 basal circumference for ORK6 was slightly greater than those listed for domestic cattle, but 6 mm less than that of aurochs (Table S10a-d).

Maxillary toothrow and individual tooth dimensions therefore indicate that although ORK6 measurements are slightly smaller than those published for aurochs, they are larger and, in particular, wider, than comparable measurements on the other LON cattle specimens.

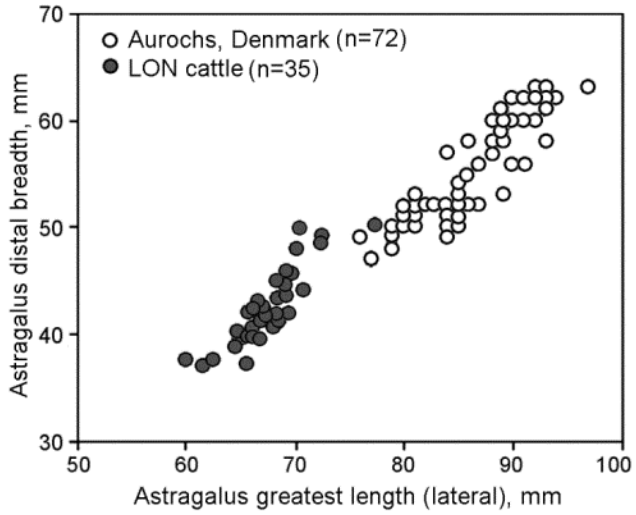
Another cattle skull, F6718, from the Structure 9 foundation course with maxillary tooth alveoli (but no teeth preserved), had an estimated tooth row length of 170 mm taken *in situ*

311 (Figure 6) and an estimated condylobasal length of 550 mm; dimensions exceeding those for
312 Neolithic domestic males and within the range for male aurochs.

313

314 Selected LON cattle post-cranial bone measurements from midden deposits dated to the Late
315 Neolithic (Moore and Wilson, 2011) were compared with those of aurochs from Denmark
316 (Degerbøl and Fredskild, 1970) in order to trace further evidence for aurochs at the site.

317 Figure 7 shows a clear separation between the astragali of aurochs from Denmark and cattle
318 from LON, with the exception of a single large specimen in the latter group that
319 conspicuously falls within the lower size range of (female) wild cattle.



320

321 **Figure 7:** Late Neolithic cattle astragalus measurements from LON and pooled male and female aurochs from
322 Denmark (Degerbøl and Fredskild, 1970).

323

324 Figure 8 shows medians for aurochs measurements for metapodial bones from Denmark are
325 greater than those from LON cattle but there is overlap at the higher range of LON cattle and
326 the lower range of the Danish aurochs, a trend also observed in bovines in Central Europe
327 (Bökönyi, 1995). This overlap is especially conspicuous in the case of metacarpals, more
328 prone to age-related mediolateral broadening (Bartosiewicz, et al., 1997).

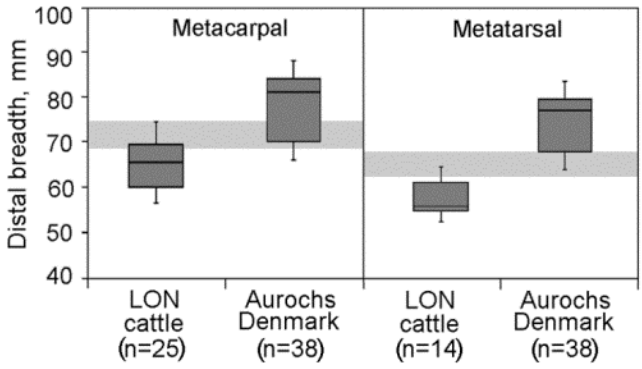


Figure 8: Metacarpal (left) and metatarsal (right) distal breadths from LON and pooled male and female aurochs from Denmark (Degerbøl and Fredskild, 1970). Graphs represent median, 25% and 75% quartile values and maximum range. The grey zones mark size overlaps between large Neolithic domestic cattle and small aurochs from Central Europe after Bökönyi (1995).

DISCUSSION

Presence/absence of aurochs

One LON cattle sample carried the hg P associated with European aurochs. Several explanations are possible: imported live aurochs; imported curated aurochs skulls; aurochs colonising Orkney naturally or progeny from European aurochs incorporated into domestic herds.

Importation of living adult aurochs to Orkney by Mesolithic or Neolithic communities would have been difficult in view of their size, strength and historically documented ferocity (Twiss, 2008). Beyond individual maternal protection, wild bovine herds tend to form circles protecting their young in the case of danger; so even encountering an unprotected calf or calves for capture would have been rare. In addition, during the Neolithic, a viable aurochs population may have competed with domestic cattle husbandry. However, capture and sea transportation is offered as an explanation for the presence of red deer in Orkney (Clutton-Brock, 1979, Stanton, et al., 2016), the Western Isles (Mulville, 2010, Serjeantson, 1990) and Ireland (Montgomery, et al., 2014) during the Neolithic.

If only aurochs skulls were imported into Late Neolithic Orkney aurochs-sized post-cranial bones should be absent from LON. There are however, a few examples to indicate that post-cranial aurochs (skeletons) of the size of pooled male and female specimens from Denmark were present in LON midden material. In addition ORK6 cattle skull (hg P) was consuming C_3 plants fertilised by manuring in a maritime environment, a similar grazing regime to those of the LON domestic cattle (hg T3). This makes it less likely that ORK6 cattle skull stems from an imported aurochs skull.

An alternative is that aurochs colonised Orkney naturally. Aurochs would not have survived the Last Glacial Maximum (LGM) but during the following warm Lateglacial Interstadial non-Arctic mammals migrated north from refuges in continental Europe, when Britain was still attached to northern Europe (Lambeck, 1995, Shennan, et al., 2000). The colder Younger Dryas followed when it is considered unlikely that non-Arctic mammals survived in Britain (Yalden, 1999). The Younger Dryas ended abruptly between 9800-9500 cal BC (Lowe and Walker, 1997) and the only opportunity for aurochs to colonise Orkney would have been before the land bridge from mainland Scotland was wholly breached. Although palaeontological evidence deems this unlikely (Shennan, et al., 2000, Sturt, et al., 2013) it cannot ultimately be precluded.

The final explanation for the presence of aurochs mtDNA in LON cattle is the integration of female aurochs into domestic cattle herds prior to arrival to Orkney. Genome sequencing of a male aurochs excavated from Derbyshire, England dated 6738±68 cal BP revealed evidence of an admixture between aurochs and the ancestors of British and Irish breeds, in contrast to those from mainland Europe (Park, et al., 2015). Wild cattle were perhaps used to improve fitness of the herd in northern climates. Incorporation of female aurochs is proposed for the small cow carrying the aurochs mtDNA variant in Neolithic Switzerland, possibly in the course of advanced management practice during the period of the secondary products

revolution (Schibler, et al., 2014). The length of the maxillary toothrow in cattle skull (ORK6) falls within the uppermost size range of Neolithic domestic cows in Britain while the same measurement estimated *in situ* on skull F6718 corresponds to the average of aurochs bulls from both Britain and Denmark might be evidence that the possible introgression event happened in the recent past.

Origin of domestic cattle

Six out of twelve cattle with hg T3 belong to the most common lineage of domestic cattle in Europe. Two further T3 variants are unique to the site of LON. In combination, the variance present is equally distant to Near Eastern (Iran) and South-eastern European, southern European, and Central-north-western European haplotype frequencies of Neolithic cattle. Unfortunately, archaeogenetic samples from the British Isles and Scandinavia are not available, at least not in statistically relevant numbers, for comparison. It is possible that individuals belonging to the dominant T3 haplotype were introduced from mainland Europe, either alongside the cattle with deviant haplotypes, or that the latter were brought from other places. This might explain the relatively high haplotype diversity, similar or higher than in Neolithic Italy, Spain, Western Anatolia and Western Europe (Scheu, et al., 2015), at a remote site on the island of Westray. The LON cattle samples were directly dated to between half to one millennium after the estimated date of introduction of farming into Orkney, suggesting they did not form a founder population. All these cattle had been raised in a similar environment, possibly Orkney. Recently, Stanton, et al. (2016) found that red deer from the Scottish Hebridean islands and Orkney probably did not originate from either mainland Scotland, Ireland or Norway; they suggest the large ungulates may have been brought to the islands by Neolithic maritime travellers from an "unknown source". Given the genetic distance of the LON cattle to known Near Eastern and European bovids, this

reinforces the complex pattern of large herbivore introductions to these archipelagos by prehistoric communities.

CONCLUSION

Cattle bone and teeth mtDNA samples from Late Neolithic Orkney displayed a diverse range of genetic profiles, perhaps indicating multiple introductions to this archipelago. Bone deposits reveal that cattle were an important resource to the Orkney Neolithic communities and may have fulfilled a symbolic role since cattle skulls, including those of males, were deposited between the inner and outer foundation stones of Structure 9 at LON. One cattle skull carried the hg P haplotype associated with European aurochs, and although several explanations for the presence of this genetic profile in Orkney are viable, isotope evidence demonstrates all sampled cattle grazed on similar pastureland that might indicate previously unrecorded evidence of aurochs/domestic cattle hybrids in northern Britain.

Acknowledgements

Historic Environment Scotland supported the archaeozoological, radiocarbon, isotope and archaeogenetic analyses for LON cattle bones. The authors would like to express gratitude to Graeme Wilson and Hazel Moore (EASE Archaeology) and Dr Jerry Herman and Dr Andrew Kitchener (National Museums Scotland).

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Supporting online material

Table S1: Primer sequences ('5-'3) used to amplify mtDNA and zfx/zfy loci.

Primer name	Locus	Sequence ('5-'3)	Position of target	Length of target (bp, excl. primer)	Reference
H1	HVR1, mitochondrial d-loop (V00654)	gcaccctaaccaaatattacaaacac	15,903-16,023	121	Bollongino (2005)
L2		gtcatgtacttgcttatatgcatggg			
H3		tatgccccatgcatataagcaa	16,041-16,152	112	
L4		cggcatggtaattaagctcgtg			
H5		taccatgccgcgtgaaacca	H5-L6: 16,185-16,271	H5-L6: 85	
L6		tgagatggccctgaagaaagaa			
L7		tccatcgagatgtcttatttaagagga	H5-L7: 16,185-16,312	H5-L7: 128	
zfx_u	Zinc finger, X chromosome (NM_177490)	agtgagtccatacacgtgtctgaca	459-486	27	Scheu, et al. (2008)
zfx_l		cgatttctgcctctactacgctat			
zfy_u	Zinc finger, Y chromosome (NM_177491)	cctctactacactaccatgaacaat	424-450	27	
zfy_l		gtcttgaccagtgagtctgtacat			

Table S2: Geographic and time bins of archaeological *Bos* samples.

Organism	Origin	Age BC	Name of bin	Number of samples	GenBank acc. codes
Bos taurus	Iran	Neolithic, 7,000–5,000	irn	10	JQ280503; JQ280506–JQ280512; JQ280514; JQ280515
	Iran, Syria	Post Neolithic, 4,000–1,400	ispn	6	JQ280501; JQ280504; JQ280505; JQ280513; JX870133;

					KF307295
	Western Anatolia	Neolithic, 6,400–5,700	wan	6	KF307310–KF307312; KF307314; KF307315; KF307317
	Southeastern Europe	Early Neolithic, 6,200–5,500	seeen	33	KF307225–KF307228; KF307249–KF307271; KF307276–KF307278; KF307281–KF307283
		Middle/Late Neolithic, 5,500–5,000	seemln	19	KF307209–KF307211; KF307223; KF307229–KF307234; KF307236; KF307237; KF307280; KF307284; KF307285; KF307287–KF307289; FJ005305
		Chalcolithic, 5,000–4,000	seec	8	KF307220–KF307222; KF307238–KF307241; KF307279
		Bronze Age, 2,700–2,200	seeba	7	KF307242–KF307248
	Southeastern Central Europe	Neolithic, 5,100–4,000	secen	19	KF307274; KF307275; KF307301–KF307309; JX870110; JX870121; JX870122; JX870127; JX870128; JX870132; FJ005306; FJ005308
	Italy	Neolithic, 6,000–5,500	itn	4	KF307296–KF307298; KF307300
	Southern France	Neolithic, 5,500–4,500	sfn	8	KF307218; KF307219; KF307224; KF307290–KF307294
	Central/Western Europe	Neolithic, 5,400–4,400	cwen	21	KF307318–KF307321; JX870112–JX870120; JX870123–JX870126;

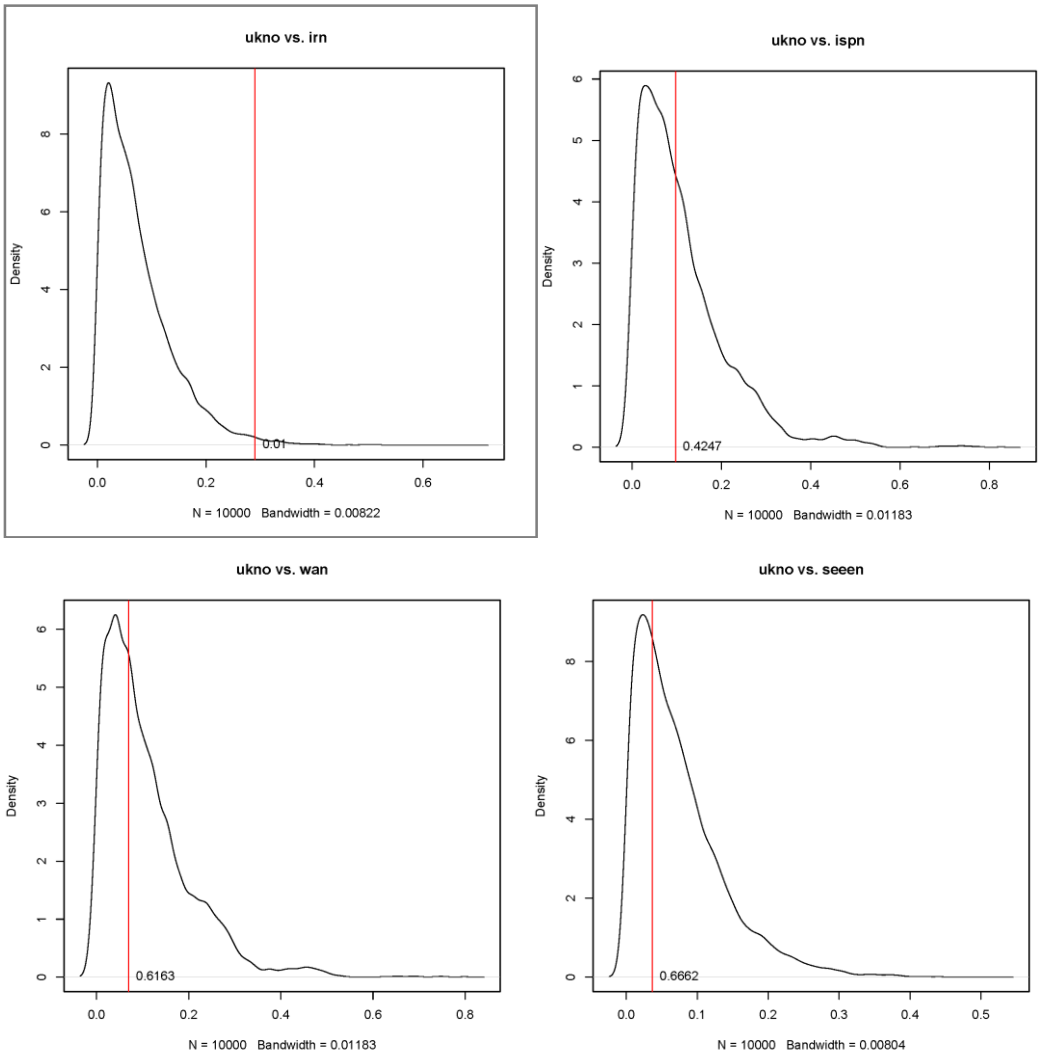
					JX870134–JX870136; DQ915555
		Post Neolithic, 4,400–2,500	cwepn	7	KF307212–KF307217; KF307322
	Central/Northern Europe	Post Neolithic, 4,400–2,500	cnepn	11	JX861235–JX861240; JX870137; JX870138; KC172647–KC172649
	UK, Orkney	Late Neolithic, 3,100–2,300	ukn_o	12	KU255585–KU255589; KU255590–KU255597
	UK, Main Land	Late Neolithic, 4,400–2,500	ukn_ml	3	DQ915527; DQ915532; DQ915576
Bos primigenius	UK			13	KU255590; DQ915524; DQ915526; DQ915528–DQ915531; DQ915533–DQ915537; DQ915558
	Central/Western Europe			9	FJ005307; DQ915522; DQ915523; DQ915541–DQ915544; DQ915552; DQ915560
	Central/Northern Europe			13	DQ915556; DQ915557; DQ915561–DQ915569; KC172646; KC172650
	Southeatern Central Europe			5	DQ915519; DQ915521; DQ915548; DQ915554; DQ915573

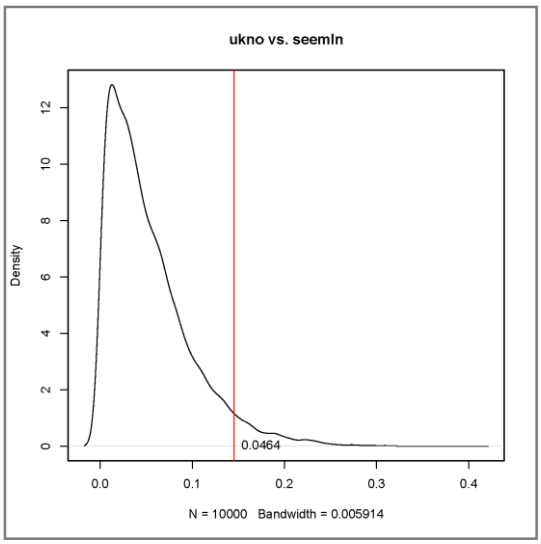
Table S3: Alignment of LON tooth and bone samples to the Bovine Reference Sequence V00654. Similarities are indicated by dots.

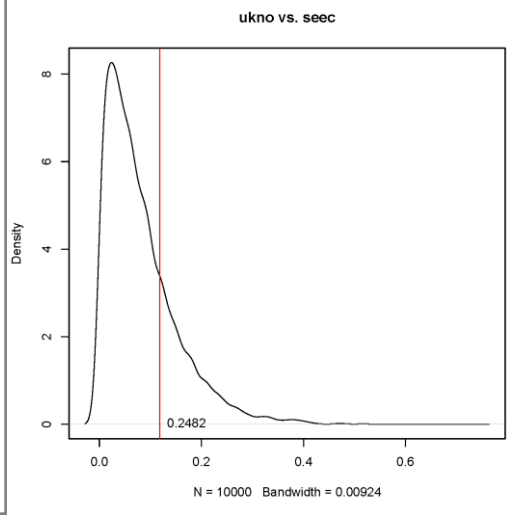
Sample	15951	15953	15994	16049	16051	16052	16058	16074	16085	16119	16122	16231	16247	16250	16255	16264	16278	Haplotype
V00654	T	C	A	C	T	C	C	T	T	T	T	C	C	A	T	G	C	
ORK1	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	T3_2
ORK2	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	T3_2
ORK3	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	T3_2

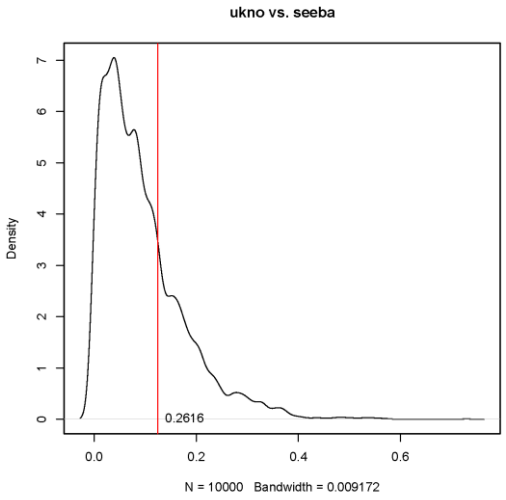
ORK4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T3_1
ORK5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T3_1
ORK6	C	G	G	T	C	.	T	C	C	.	C	T	.	.	C	A	Y	P
ORK7	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	T3_1
ORK8	.	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	T3_3
ORK9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T3_1
ORK10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T3_1
ORK12	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	T3_2
ORK14	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	T3_2
ORK15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T3_1

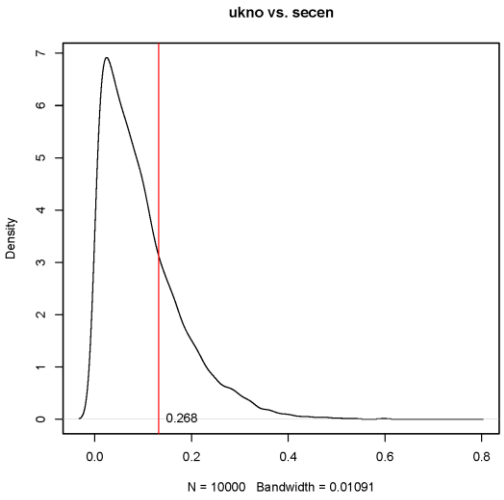
Figure S4: Density plots of 10,000 permutation test to reject statistical bias introduced by uneven samples sizes of geographical and/or time bins, based on haplotype diversity. Comparable bins are framed. Significance level < 0.05.

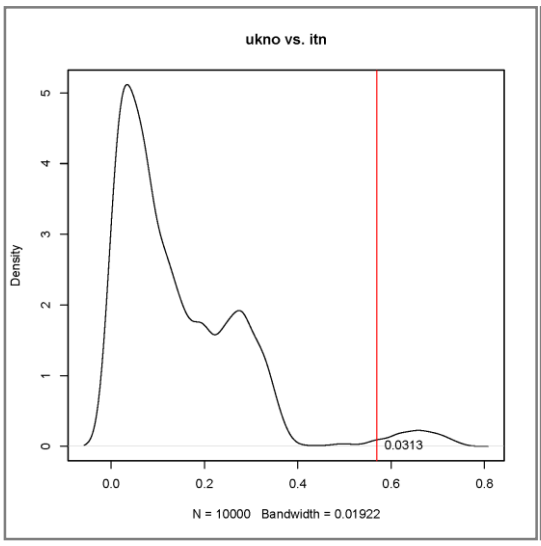


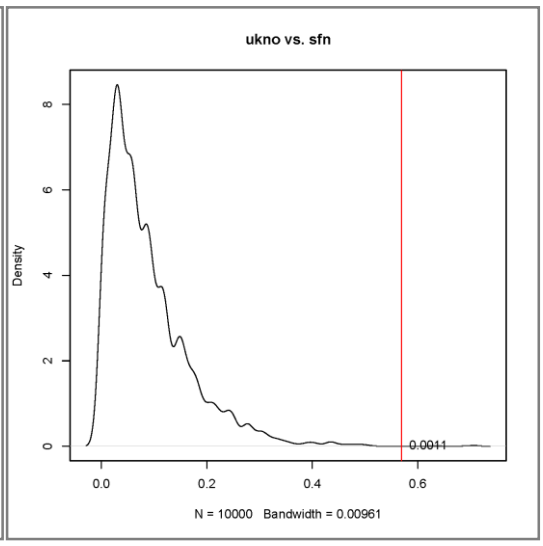

ukno vs. seemln
Density
0.0464
N = 10000 Bandwidth = 0.005914


ukno vs. seec
Density
0.2482
N = 10000 Bandwidth = 0.00924


ukno vs. seeba
Density
0.2616
N = 10000 Bandwidth = 0.009172


ukno vs. secen
Density
0.268
N = 10000 Bandwidth = 0.01091


ukno vs. itn
Density
0.0313
N = 10000 Bandwidth = 0.01922


ukno vs. sfn
Density
0.0011
N = 10000 Bandwidth = 0.00961

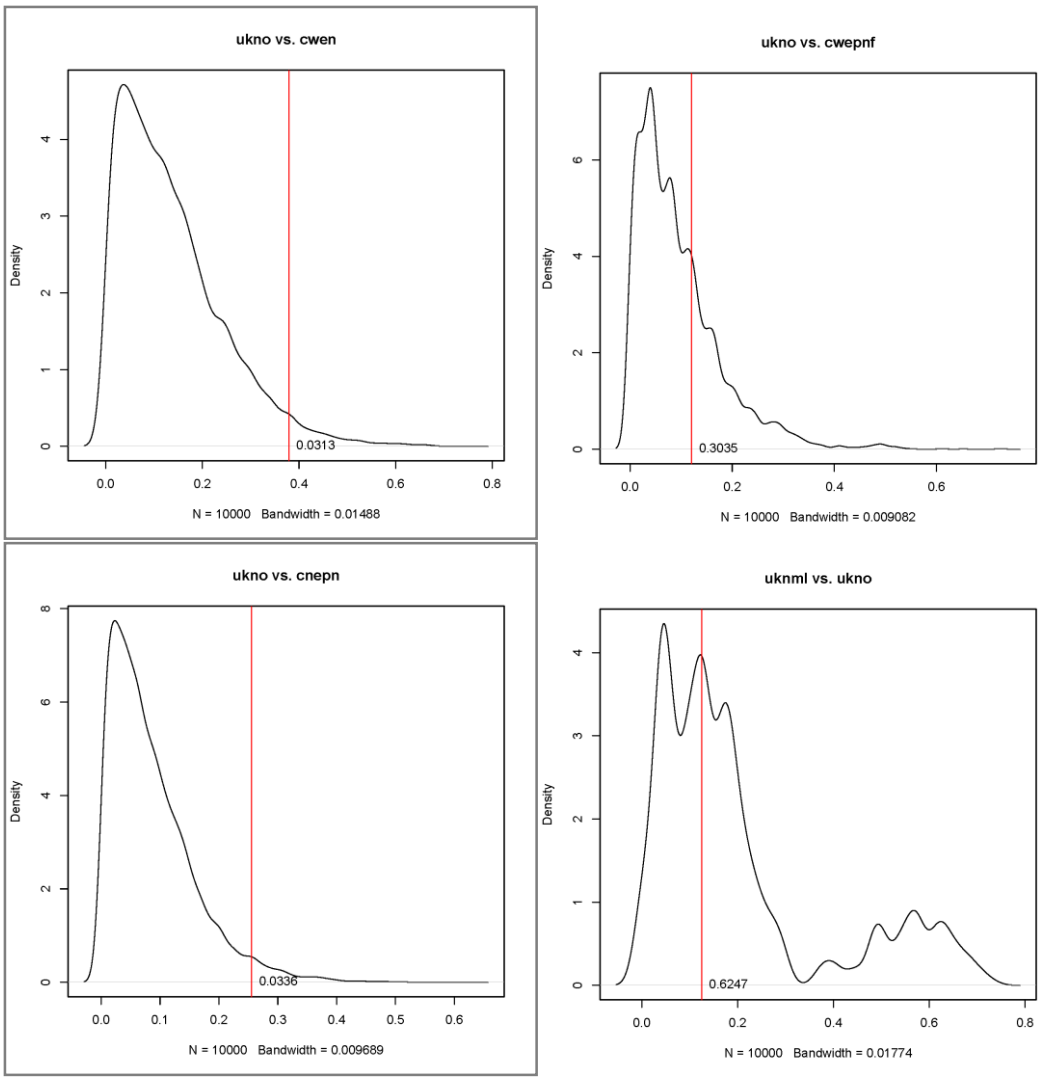


Figure S5: The contribution (loading) for the PCA graph of each haplotype is presented by bar charts for component 1 (left) and 2 (right). Singleton haplotypes were excluded. Genebank accession codes (representative) for haplotypes: Bt_ht4 = KF307287; Bt_ht5 = KF307295; Bt_ht8 = KF307311; Bt_ht16 = KF307211.Haplotypes unique to LON are Bt_ht30 (KU255586) and Bt_ht31 (KU255588).

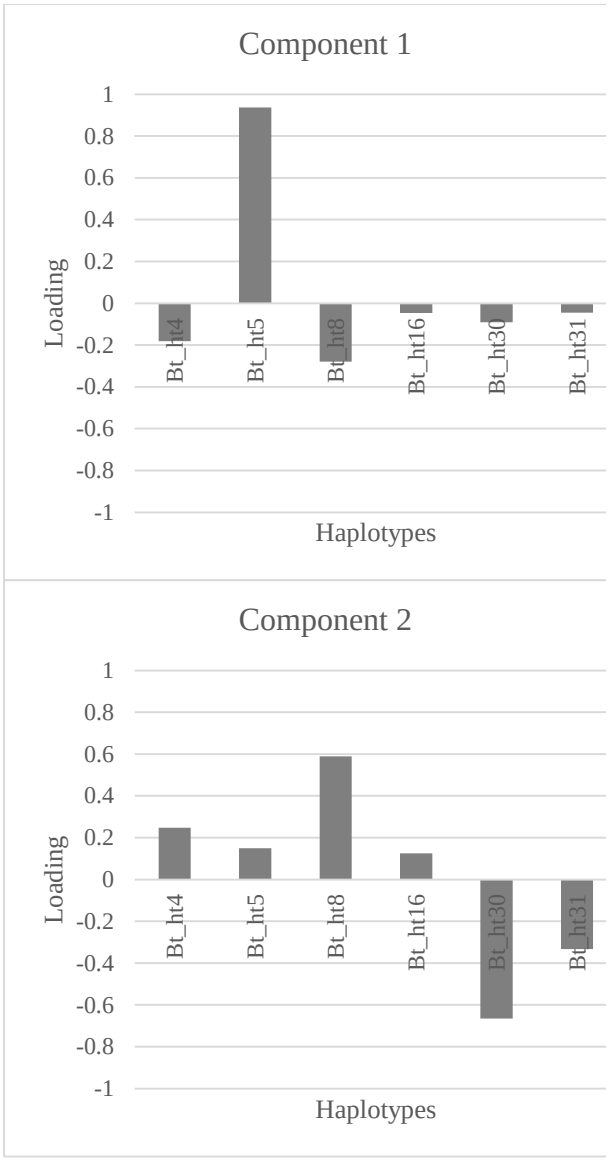

Component 1
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Loading
Bt_ht4
Bt_ht5
Bt_ht8
Bt_ht16
Bt_ht30
Bt_ht31
Haplotypes
Component 2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Loading
Bt_ht4
Bt_ht5
Bt_ht8
Bt_ht16
Bt_ht30
Bt_ht31
Haplotypes

Table S6: Stable isotope results. Given are DNA laboratory code and code of the Scottish Universities Environmental Research Centre (SUERC), sampled skeletal elements as well as $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values and ratios, as well as mitochondrial haplogroup.

Lab code	SUERC Code	Bone	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	%C	%N	%S	C:N	C:S	N:S	mtDNA hg
ORK1	51170	P^3	–21.3	5.4	16.8	39.1	13.7	0.21	3.3	487	148	T3
ORK2	51171	M_3	–20.4	7.4	19.1	23.8	8.4	0.17	3.2	373	117	T3
ORK3	51172	M^1	–21.3	6.7	19.2	26.4	9.3	0.17	3.2	407	125	T3
ORK4	51173	P^2	–21.4	6.0	18.3	25.7	9.1	0.17	3.2	402	124	T3
ORK5	51174	M^2	–21.1	6.7	19.9	27.6	9.8	0.17	3.2	451	139	T3
ORK6	51175	P^3	–21.1	5.6	18.3	38.0	13.5	0.20	3.2	467	144	P
ORK7	51176	M_1	–21.7	7.3	19.6	27.5	9.9	0.17	3.2	432	136	T3
ORK8	51182	M^3	–21.6	7.4	20.8	39.4	14.1	0.22	3.2	454	141	T3
ORK9	51180	Radius	–21.4	5.5	19.4	23.6	8.2	0.16	3.3	386	117	T3
ORK10	51181	Radius	–21.6	6.3	18.7	29.4	10.4	0.16	3.3	461	142	T3
ORK12	51183	M/t	–21.4	5.3	17.4	30.7	10.9	0.16	3.2	488	152	T3
ORK13	51184	Humerus	–21.6	5.1	17.7	30.4	10.5	0.17	3.3	446	135	-
ORK14	51185	Phalanx	–21.3	5.0	17.6	21.0	7.4	*0.13*	3.3	425	129	T3
ORK15	51186	M/t	–21.3	6.0	19.0	18.8	6.4	*0.12*	3.3	*284*	*86*	T3

Table S7: Radiocarbon dates for cattle bone and teeth with mtDNA results. Given are DNA laboratory code and code of the Scottish Universities Environmental Research Centre (SUERC), sampled skeletal elements as well as uncalibrated and calibrated ^{14}C results, calibrated using OxCal 4.2 (Bronk Ramsey 2009) and the Internationally-agreed terrestrial calibration curve (IntCal13) of Reimer, et al. (2013).

Lab code	SUERC Code	Bone	^{14}C age (years BP)	Calibrated date (cal BC 95.4%)	Calibrated date (cal BC 68.2%)
ORK1	51170	Maxillary P^3 tooth	3973 ± 35	2578–2349	2566–2466
ORK2	51171	Mandibular M_3 tooth	4236 ± 36	2916–2694	2905–2764
ORK3	51172	Maxillary M^1 tooth	4256 ± 35	2923–2704	2911–2874
ORK4	51173	MaxillaryP^2 tooth	4228 ± 35	2911–2681	2900–2762
ORK5	51174	Maxillary M^2 tooth	4299 ± 35	3012–2879	2927–2884
ORK6	51175	Maxillary P^3 tooth	4217 ± 35	2905–2678	2894–2712
ORK7	51176	Mandibular M_1 tooth	3970 ± 35	2577–2348	2566–2464
ORK8	51180	Radius	4039 ± 35	2835–2472	2618–2539
ORK9	51181	Radius	4158 ± 35	2880–2626	2871–2678
ORK10	51182	Maxillary M^3 tooth	4158 ± 35	2880–2626	2871–2678
ORK12	51183	Metatarsus	4338 ± 35	3082–2893	3011–2904
ORK13	51184	Humerus	4064 ± 35	2853-2486	2833-2497
ORK14	51185	Proximal phalanx	4188 ± 35	2891–2639	2884–2698
ORK15	51186	Metatarsus	4270 ± 35	3008–2711	2911–2881

Table S8: Cattle maxillary toothrow length (P^2–M^3, mm). Measurement 20, figure 8d (von den Driesch, 1976). Abbreviations: A=adult, SA=subadult, ND=not identifiable

Lab. code	LON Skull Find No	Toothrow length (mm)	Age (Simonds 1854)	mDNA status	Sex
ORK1	F4257	132	A	T3/2	ND
ORK3	F6693	130	A	T3/2	Male?
ORK4	F4462	137	A	T3/1	ND
ORK5	F4460	123	SA	T3/1	Male?
ORK6	**F4459**	142	A	P	Female

Figure S9: Buccal crown height of maxillary molars from cattle skulls.

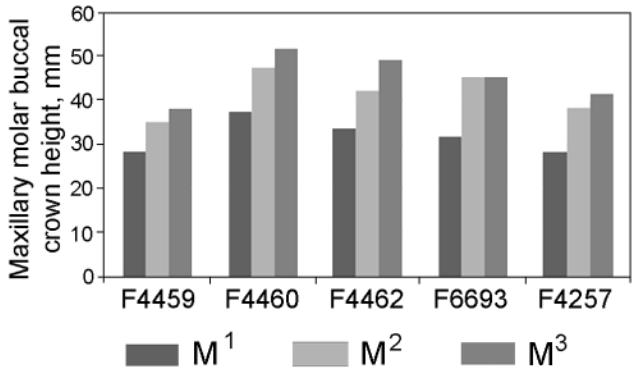


Table S10a: Dimensions of maxillary 1st molar (left, in mm).

Lab code	LON Skull Find no	Occlusal length (mm)	Occlusal breadth (mm)	Cervical length buccal (mm)	Ratio occlusal breadth/ length	Circumference of base (mm)
ORK1	F4257	24.4	21.0	18.6	86%	83 (Right)
ORK3	F6693	26.7	18.0	18.6	67%	
ORK4	F4462	26.3	18.5	18.6	70%	
ORK5	F4460	29.0	19.8	18.4	68%	
ORK6	**F4459**	24.8	22.2	21.5	90%	93

Table S10b: Dimensions of maxillary 2nd molar (left, in mm).

Lab code	LON Skull Find no	Occlusal length (mm)	Occlusal breadth (mm)	Cervical length buccal (mm)	Ratio occlusal breadth/ length	Circumference of base (mm)
ORK1	F4257	28.2	21.0	23.6	74%	89(Right)
ORK3	F6693	31.0	19.1	22.2	62%	93(Right)
ORK4	F4462	29.9	20.4	22.7	68%	
ORK5	F4460	31.5	19.9	22.4	63%	91
ORK6	**F4459**	30.5	24.0	24.8	79%	96

Table 10c: Dimensions of maxillary 3rd molar (left, in mm).

Lab code	LON Skull Find no	Occlusal length (mm)	Occlusal breadth (mm)	Cervical length buccal (mm)	Ratio occlusal breadth/ length	Circumference of base (mm)
ORK1	F4257	28.0	18.4	27.5	66%	93(Right)
ORK3	F6693	28.6	18.2	24.8	64%	93(Right)
ORK4	F4462	27.1	18.3	24.3	68%	
ORK5	F4460	29.3	18.6	26.3	63%	94
ORK6	**F4459**	31.3	23.3	29.9	74%	101

Table S10d: Dimensions of maxillary 4th premolar (left, in mm).

Lab code	LON Skull Find no	Occlusal length (mm)	Occlusal breadth (mm)	Cervical length buccal (mm)	Ratio occlusal breadth/ length
ORK1	F4257	19.4	17.8	14.6	92%
ORK3	F6693	19.3	16.9	13.6	88%
ORK4	F4462	19.5	18.0	12.6	92%
ORK5	F4460	20.5	17.4	12.9	85%
ORK6	**F4459**	19.2	21.2	15.0	110%