An early date for cattle from Namaqualand, South Africa: implications for the origins of herding in southern Africa

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When did cattle come to South Africa? Radiocarbon dates on a newly found cattle horn indicates a time in the early first millennium AD. In a study of the likely context for the advent of cattle herding, the authors favour immigrants moving along a western route through Namibia.

Keywords: South Africa, cattle, early herding, dispersal routes, Namaqualand

Ancient DNA analysis

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In these analyses we have employed the Ion Torrent™ sequencing platform, which is one of the systems developed to sequence single strands of DNA, collectively known as Next Generation, or high-throughput, sequencing. All ancient DNA extraction and preparation of Ion Torrent sequencing libraries prior to PCR amplification was conducted in the University of Otago ancient DNA facility, New Zealand. Multiple methods were employed to avoid contamination by modern DNA or PCR amplicons (Knapp *et al.* 2012). In particular, the laboratory is never entered after visiting a modern laboratory without first showering, washing hair and changing into freshly laundered clothes. No equipment, reagents or samples are taken from the modern to the ancient laboratory. To prevent introducing contaminating DNA from clothing, street clothes and shoes are removed before entering the laboratory and workers change into dedicated laboratory scrubs, hairnets, coveralls, face-masks, laboratory-only shoes and two pairs of gloves. The laboratory is maintained under HEPA-filtered positive pressure, and ultraviolet lights are used to degrade DNA traces on laboratory surfaces whenever the space is unoccupied.

DNA was extracted from two specimens in Namaqualand, South Africa: a horncore from KN2005/041 near Koingnaas and a fragment of maxilla from Reception Shelter near Vredendal. A total of 2.86g of bone from the horncore and 1.1g of tooth extracted from the maxilla were ground using a sterile mortar and pestle, and DNA extraction was undertaken with using a silica/guanidinium thiocyanate protocol (Rohland & Hofreiter 2007). A negative extraction control was processed in parallel with each specimen.

Libraries for the Life Technologies Ion Torrent sequencing platform were produced directly from the aDNA extract using the Ion Plus Fragment Library Kit (Catalogue number 4471252) and the Ion Xpress™ Barcode Adapters with a slight modification to the manufacturer's protocol. We used 0.5uL of the barcoded A and P1 adapters, rather than the 2uL specified, to minimise the frequency with which the A and P1 adapters ligated to each other.

Newly prepared sequencing libraries were subjected to quantitative PCR (qPCR) on a Stratagene MxPro 3000P system using SYBR Green dye (ABI) to determine the number of PCR cycles necessary to reach their amplification plateau and thereby avoid

the production of chimeric molecules (Meyerhans *et al.* 1990; Odelberg *et al.* 1995; Judo *et al.* 1998; Thompson *et al.* 2002). The Ion Torrent libraries, and their associated negative controls, are therefore 'immortalised' by PCR amplification (Amplitaq Gold, ABI) using only the number of cycles determined necessary to reach plateau. Amplification products from the qPCRs were run on 2% agarose gels to check for the presence of sequencing libraries, evidence by a smear of different length DNA molecules, and the absence of strong adapter dimers. Immortalised libraries were purified with MinElute purification columns (Qiagen) following the manufacturer's protocol. Purified, immortalised libraries were again subjected to qPCR to determine the number of PCR cycles necessary to reach their amplification plateau.

Employing a few modifications from the protocol outlined by Maricic *et al.* (2010) sequencing libraries were enriched for the mitochondrial genome by in-solution hybridisation capture. The modifications were that each library was enriched independently, and pooled only after quantification by qPCR on a Stratagene MxPro 3000P using SYBR green (ABI). Further, libraries were eluted from the streptavidin beads by heat treatment instead of sodium hydroxide. Pooled, barcoded libraries were sequenced on the Life Technologies Ion Torrent sequencing platform according to the manufacturer's protocol.

We mapped the individual reads against a reference sequence (GenBank JN869312 for the gemsbok and V00654 for the cattle) using Bowtie 2 (http://bowtie-bio.source-forge.net/bowtie2/index.shtml). We then applied the Samtools software package (Li *et al.* 2009) to remove clonal sequence reads and call the consensus sequence and variants such as single nucleotide polymorphisms (SNPs). The SNPs were called and filtered using bcftools (part of the Samtools software package). To confirm the authenticity of our ancient DNA data we used the perl script mapdamage 0.3.5 (http://geogenetics.ku.dk/publications/mapdamage/) (Ginolhac *et al.* 2011). This tool allows us to characterise known ancient DNA damage such as the increase C–T mutations at the 5-prime end of the reads. These patterns of deamination damage are characteristic for ancient DNA and can therefore be used to distinguish aDNA from modern contamination. Neither negative control contained any reads that mapped to

cattle, gemsbok or human DNA, indicating that our reagents were not contaminated and that we did not cross-contaminate during processing. Both specimens provided some recoverable DNA, although the organic preservation in the horn core was poor and we were able to recover only 2.64% of the mitochondrial genome. The few recovered sequences are consistent with the mitochondrial genome of *Bos taurus*. From the maxilla, however, we were able to recover 88.87% of the mitochondrial genome, with each base sequenced on average 46 times. The recovered mitochondrial genome is that of a gemsbok (*Oryx gazella*). It has been deposited in GenBank (Accession Number KC282640).

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