

Discovering genetic variance in ancient sheep from three Polish Bronze Age sites, gives insight into human trading routes

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Introduction

Domesticated sheep (*Ovis aries*) are one of the most important catalysts for recent human adaptation since the Neolithic. Sheep provided Neolithic humans stability through a constant supply of meat, wool, and milk (Kijas et al., 2012). This allowed Neolithic humans to grow exponentially in numbers, extend life expectancy, and have time to explore art and tool making (Weisdorf, 2005). Performing a genetic analysis (comparing the amount of mutations between organisms) can establish evolutionary events such as selection, migration, and population growth. The purpose of this study was to perform a phylogenetic analysis on 14 ancient sheep samples from three Bronze Age sites (3650–3100 BCE) in Poland (Brononice, Niedzwiedz, and Zawarza). The aim was to draw conclusions about the herding human populations that inhabited those sites, such as trade and migration routes. The control region of the mitochondrial DNA (mtDNA) was analyzed since mutations in this area rarely affect the survival of the individual and tend to accumulate over time, as they are not affected by natural selection. It is assumed that the more mutations (differences between samples) found, the more time/evolutionary distance there is between organisms (Kimura, 1983). A Haplogroup is a categorization of DNA sequences that are very similar or nearly identical to each other when compared to other sequences. Haplogroups are useful in indicating evolutionary history that the individuals have in common (Tapio et al., 2006). Haplotypes are subgroups within a Haplogroup and are represented by circles in this analysis. This study aimed to determine the Haplogroup of 14 ancient sheep remains found on three archaeological sites in Poland. The mitochondrial genome of sheep has been categorized into five major Haplogroups – A, B, C, D, and E (Tapio et al., 2006). Haplogroups A and B are found in higher frequency in Europe (Dymova et al., 2017). Since the ancient samples are from European origin, we expected them to cluster within these groups when compared to a sheep dataset that includes modern members of all five Haplogroups. This finding would indicate intracontinental exchange of farming goods between European Bronze Age populations.

Procedures

The ancient samples were retrieved by a group of archaeologists, which included Milsaukas and Kruk, from the State University of New York at Buffalo. Ancient DNA was extracted and the hypervariable region I (HVI) was amplified and sequenced at Binghamton University in N.Y. by Dr. Jennnifer Kennedy and Dr. Alondra Díaz-Lameiro. A dataset was constructed to determine the Haplogroup of the ancient samples. Similar sequences were retrieved using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) website. BLAST searched online databases, such as GenBank, for

Procedures (cont.)

DNA sequences that are very similar to the ancient sheep samples. For the first dataset 436 modern samples were retrieved and attributes such as country of origin were documented. The dataset was composed of 2% African, 14% Asian, and 84% European sheep. A second dataset of 297 modern samples was built with individuals belonging to Haplogroup B. This dataset was 3% African, 5% Asian, and 92% European sheep. Haplogroups D and E were not present in the samples. The dataset was then imported into MEGA 7 (Kumar et al., 2018) for alignment using the MUSCLE algorithm (Edgar, 2004). Once the dataset was aligned, a sequence fragment of 674 base pairs was used for analysis. Sequences were then grouped into haplotypes, based on their differences using DNAsp software (Librado, 2009). The alignment was then exported into Network 5 (Bandelt, 1999) where a median-joining network was calculated with the complete data set (Figure 1). For clarity and definition, a network was calculated with the second dataset which included the sequences that were most closely related to the ancient sheep samples (Figure 2). Both networks were color-coded based on country of origin, the size of the circles corresponds to the number of samples that shared a particular haplotype, and physical distance between circles represents the genetic distance between haplotypes.

Results (cont.)

The first network (Figure 1) included sheep samples from Europe, Asia, and Africa, and clustered into three distinct Haplogroups, A, B, and C. The ancient sheep samples reside in the same maternal lineage as haplotypes from Spain and Greece, along with other European and African haplotypes, such as Kenya and the United Kingdom. Asian haplotypes resided mostly in Haplogroup C, with some in Haplogroup A.

Most of the ancient sheep samples (Figure 2), in grey, are between one and two mutations away from the center haplotype (largest circle) which is prevalent across Europe with a 67% presence in Spain and Greece as compared to the rest of the European countries. This suggests that Haplogroup B might have originated in the Mediterranean, with wild sheep breeds such as the Awassi (Georgoudis, Rosati, & Mosconi, 2005), and then spread, most likely through trade. Haplogroup B consisting of 3% African haplotypes suggested sheep from that group migrated to Africa.

Conclusions

All 14 of the ancient samples from the Polish Bronze Age clustered within Haplogroup B (left cluster in Figure 1 and Figure 2). They share haplotypes with European countries, and do not show relationships with any Asian samples. These results suggest that the individuals from the three villages (Brononice, Niedzwiedz, and Zawarza) traded sheep locally or within Europe, and were most likely not involved in major trade routes spanning other continents, at least on the female (ewe) side. This study could not discard the possibility of intercontinental trade of males (rams) in the European Bronze Age, as only the maternal ancestry was analyzed.

Haplogroup A (bottom right cluster in Figure 1) seems to be the most ancient, since there is a larger variety of haplotypes within this group (European, African, and Asian). The fact that Haplogroups B and C seem to emerge from Haplogroup A, also points to Haplogroup A as a more ancestral line (Dymova et al., 2017), although more data needs to be analyzed in order to corroborate this finding. The three sites do not show any clustering pattern, and are spread over the network.

Results

| Legend | |
|--------------|-------------|
| Country | Color |
| Albania | Dark Blue |
| China | Orange |
| Cyprus | Light Blue |
| Egypt | Dark Red |
| France | Blue |
| Germany | White |
| Greece | Light Blue |
| Hungary | Dark Green |
| Italy | Purple |
| Kenya | Pink |
| Poland | Pink |
| Ancient | Grey |
| Portugal | Olive Green |
| Romania | White |
| Saudi Arabia | Light Green |
| Scotland | White |
| Spain | Yellow |
| Turkey | Red |
| U. Kingdom | Cyan |

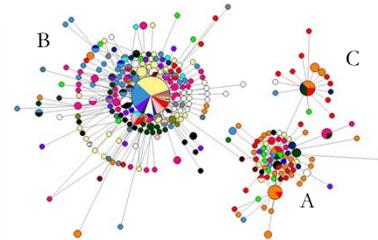


Figure 1 (above): Median-joining network calculated from a dataset of $N = 422$ modern sheep samples from European, African, and Asian continents, plus 14 ancient sheep samples from three Bronze Age sites in Poland. Each color represents a country of origin. Each ring of circles is one mutation from the center sequence. The larger the physical distance, the more mutations/differences between the individuals.

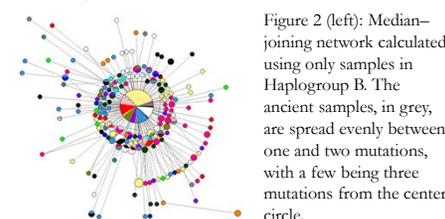


Figure 2 (left): Median-joining network calculated using only samples in Haplogroup B. The ancient samples, in grey, are spread evenly between one and two mutations, with a few being three mutations from the center circle.

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