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Elucidating recent history by tracing genetic affinity of three 16th century miners from Sweden.

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KEYWORDS: ancient DNA, mobility, forced labor

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ABSTRACT

Objectives: Sala silver mine in central Sweden was an important manufacturer of silver from at least the 16th till the early 20th century, with production peaking in the 16th, mid 17th and 19th centuries. The job opportunities offered by the mine drew people to the area resulting in the development of a small township with associated cemetery in the vicinity of the mining center. The cemetery served people affiliated to the mine for around 150 years. Written sources reveal that common convicts and war prisoners from the numerous wars fought by Sweden during the time were exploited in the mine, and some of them were likely buried on the cemetery. The cemetery has been excavated on several occasions and the recovered human remains were divided into two different groups based on burial custom, demography and biochemical results. One group was believed to contain war prisoners and the aim of this study was to produce and interpret genomic data from these individuals to test the hypothesis regarding their origins.

Materials: Dental material from seven different individuals was sampled. Results: Three of the analyzed teeth contained sufficient amounts of indigenous human DNA allowing for generation of genomic sequence data with genome coverages of 0.04, 0.19 and 0.83. Discussion: The results show that despite this apparent heterogeneity the three individuals fell within genetic variation of modern and contemporary Swedes yielding little support to their hypothetical foreign origin. However, due to the lack of contemporary or modern Danish genomic data we cannot exclude the Danish origin of these individuals.

INTRODUCTION

Silver mining constituted an important contribution to the Swedish economy during several centuries. The most important mine was located in Sala in east central Sweden (Figure 1). The earliest records mentioning an operational mine date to the beginning of the 16th century and
before abandonment of the mine in 1908, approximately 450 tons of silver were extracted from it
(Jansson and Geovetenskaper, 2007; Norberg, 1978). A village, Salberget, including a small
class. The cemetary of the church was used for only
150 years between the end of the 15th century to mid-17th century (Bäckström and Price, 2016;
Price et al., 2017). The site was excavated in 2004, 2008, 2009 and 2011, revealing in total 102
skeletons in 93 graves (Bäckström et al., 2009; Bäckström and Ingvarsson-Sundström, 2014,
2010; Onsten-Molander et al., 2005). The cemetary is believed to have been used for all social
groups in the village and mine, including local families, miners and other workers, foreign
specialists, but also forced labor in the form of prisoners of war and individuals convicted of
various crimes. Landless manual workers came from Sweden and present day Finland; the
specialists arrived from Germany, the Netherlands and Austria, and the prisoners, according to
the historical records, originated in Denmark, Russia and Poland (Boëthius, 1951; Eriksson et al.,
2003; Frankius, 2008; Norberg, 1978). During the excavations, two different types of burials
were discovered which are believed to mirror the social hierarchy. The first type consisted of 84
coffin-burials with 80 skeletons in which men, women and children were buried in shrouds. A
majority of the coffins contained single burials. Only three cist graves contained more than one
individual; one cist held two children and two cists contained an adult male and a child). The
second type containing only men (22 individuals, primarily younger men), were nine earthen
burials located primarily in the southern part of the cemetary. Most of the graves in the latter
category were multiple inhumations containing between two to eight individuals interred in their
everyday clothes (Bäckström and Ingvarsson-Sundström, 2010). The men in latter group show a
high frequency of perimortem fractures and one individual was buried with an iron shackle
around the neck (Bäckström and Ingvarsson-Sundström, 2010; Bäckström and Price, 2016). Two
isotope studies, using strontium (Sr), oxygen (O) and lead (Pb), have demonstrated significant
differences in values between the individuals associated with the two burial types (Bäckström
and Price, 2016; Price et al., 2017). The sampled skeletons from coffins exhibit values consistent with being local, perhaps families, from the Sala area, while the isotope values from men in the earthen burials are consistent with non-local origin. Due to the simple burial type, the many perimortem fractures, finding of the man with the iron collar and the isotopic values, it has been suggested that the men in the earthen burials could be identified as the prisoners of war forced to work in the mine (Boëthius, 1951; Eriksson et al., 2003; Norberg, 1978; Price et al., 2017). Here genome-wide sequence data was generated for three individuals in earthen burials in order to explore their genetic relationship to modern-day European populations. Seven skeletons were selected for the analyses. The sampling strategy focused primarily on investigating mobility in the mining community by investigating correlation between Sr-analysis results and genetic affinities of individuals. Lower Sr values around 0.712 earthen graves were linked to the southern Baltic region and higher Sr values of around 0.721 (coffin graves) were interpreted as more 'local'. Therefore, the individuals picked out for DNA analyses consisted of four skeletons from graves without coffins with lower Sr values (Sk6854, Sk6866, Sk6990, Sk6994), and three skeletons from coffin graves with higher Sr-values (Sk5498, Sk7031 and Sk7980). Only three skeletons with lower Sr-values contained preserved DNA, rendering this comparison impossible.
MATERIALS AND METHODS

The anthropological analysis of the skeletons from Salberget involved international standard techniques (Bäckström and Price, 2016). Samples for the genomic analysis were taken from teeth in the mandible of seven men from the earthen burials. Three individuals generated enough DNA for a continuing productive analysis (Table 1). One of the men (Sk6866) was buried together with seven other individuals (Grave 6826) (Bäckström and Ingvarsson-Sundström, 2010). The other two men were found together in a double grave (Grave 6975), where one of the individuals (Sk6990) was buried on top (in the arms) of the other (Sk6994) (Figure 2). All three were buried according to the Christian tradition; on their backs with their heads to the west.

Extraction of DNA and preparation of libraries
All sample preparations were performed in dedicated clean-room facilities in the ancient DNA laboratory at the Archaeological Research Laboratory, Stockholm University. Teeth were wiped (decontaminated) with 1% sodium hypochlorite solution, ddH$_2$O and 70% EtOH, and UV-irradiated (254nm wave length) at approximately 1 J/cm$^2$ per side. Dentine powder was extracted in a dead-air cabinet using a Dremel drill at the lowest possible rpm (5000rpm).

DNA was extracted following previously published protocols (Svensson et al., 2007; Yang et al., 1998). In brief, dentine powder was mixed with lysis buffer containing 0.45M EDTA (pH 8), 1M Urea, and 100µg proteinase K, and incubated with constant agitation for at least 18 hours at 37°C until fully dissolved. DNA was concentrated on Amicon Ultra-4 columns (Merck Millipore) and purified on MinElute silica columns (Qiagen) following manufacturer recommendations but with the addition of a second wash step. Purified DNA was eluted in 100µL EB buffer (Qiagen). We included two negative (blank) DNA extractions for every eighth bone sample.

Double stranded DNA sequencing libraries were prepared from 20µL DNA following Meyer & Kircher (2010). Libraries were PCR amplified, pooled at equimolar concentrations, and purified as described in Günther et al. (2015). Library pools were shotgun-sequenced on the Illumina HiSeq-2500 platform at the SciLife DNA sequencing facility, Stockholm.

Sequence analyses

Sequence data was analyzed following previously published procedures (Günther et al., 2015; Omrak et al., 2016). In summary, reads were de-multiplexed based on their respective index sequences (Meyer and Kircher, 2010). Pair-end reads were merged, trimmed and mapped to the human reference genomes, build 36 and 37, with BWA v. 0.7.13 (Li and Durbin, 2010) while the PCR duplicates were removed with FilterUniqueSAMCons.py (Kircher, 2012). The presence of
3’ and 5’ cytosine deamination patterns characteristic of ancient DNA (Briggs et al., 2007; Hansen et al., 2001; Hofreiter et al., 2001; Orlando et al., 2011; Sawyer et al., 2012) was estimated using PMDtools (Skoglund et al., 2014). Additionally, levels of contamination were estimated based on the analyses of polymorphic site distribution in mitochondrial sequences (Green et al., 2008). Individuals’ molecular sex was estimated using the method by Skoglund and colleagues (2013). The consensus sequences for the mitochondrial genomes were called using mpileup and vcfutils.pl (vcf2fq) from the samtools package (Li et al., 2009). Only reads with a minimum mapping score of 30 and a minimum base quality of 30 were used to call confident bases for the final consensus sequences. We identified the haplogroups using HAPLOFIND (Vianello et al., 2013) and PhyloTree Build 17 (18th February 2016)(van Oven and Kayser, 2009). The Y chromosome sequences were filtered out using mpileup from the samtools package (Li et al., 2009). The pileup file was then merged with both the PyloTree Y haplogroup definitions (van Oven et al., 2014) as well as the ISOGG database v. 04/2016 (http://isogg.org/tree)(International Society of Genetic Genealogy, 2017). For population based analyses the genetic data from the investigated individuals was merged with the reference population panel of 21 population samples from the Human Origins (HO) dataset (Lazaridis et al., 2014; Patterson et al., 2012), and 13 populations from Estonian Biocentre Human Genome Diversity Panel (EGDP) (Pagani et al 2016). The Human Origins dataset was additionally merged with ancient Swedish reference consisting seven 17th century unpublished genomes from southern Sweden (in prep.). The reference ancient genomes were all males sequenced between 0.38x – 1.1 x genome coverage. Only positions with minimum base and mapping qualities of 30 were used in the analyses. In order to visualize the relationship between the ancient individuals and modern reference populations we performed principal component analyses (PCA) using EIGENSOFT v.6.0.1 (Patterson et al., 2006). Furthermore, to obtain information on genetic affinities between the three individuals and the modern populations, we performed f3-outgroup
statistics using qp3Pop v. 204 (Patterson et al., 2012; Raghavan et al., 2014) and $D$-statistics which were calculated using qpDstat of ADMIXTOOLS (Durand et al., 2011; Patterson et al., 2012). All analyses were performed with exclusion of transition sites and using pseudo-haploid genomes. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) (Lampa et al., 2013) under the following projects: b2013240, b2015307 and b2016056.

**RESULTS**

We generated genomic sequence data from three individuals from Sala silvergruva (Sk6866, Sk6990, Sk6994) to 0.04, 0.19 and 0.82-fold genome coverage. Basic sequence statistics are listed in Table 1. All individuals were males and all show cytosine deamination patterns typical of ancient DNA, ranging from 8-12% increase in $C>T$ transitions at 5’ ends of sequenced DNA fragments, while contamination levels were estimated to range between 1.29% – 2.08%.

The numbers of SNPs supporting each called mitochondrial haplotype are 51 for individual Sk6866, 53 for individual Sk6994, 56 for individual Sk6990. The calls were recorded as deviations from the Reconstructed Sapiens Reference Sequence (RSRS) and the obtained haplotypes are listed in Table 1 (Behar et al., 2012). The mitochondrial lineage U5b2a1a1 found in individual Sk6866 is characteristic of central and eastern European populations (Malyarchuk et al., 2010). Haplogroup I1a1b, found in individual Sk6994, and T2b21b, found in individual Sk6990, are common in north Europe (Behar et al., 2012). Only one Y chromosome haplogroup assignment was possible using haplogroup definitions from PhyloTree: individual Sk6990 was assigned to subclade R1b1b (R-P297) which is a common haplogroup in Western Europe (Myres et al., 2011). The other individuals were assigned their respective Y haplogroups using ISOGG haplogroup definitions. Individual Sk6866 belonged to Y chromosome lineage R1b1a, while SK...
6994 was a carrier of I2a2a. The subclade R1b1a is the most frequent haplogroup in Eurasia (Balaresque et al., 2010) while I2a2a (formerly I1c) is widespread throughout Europe with highest frequencies in Germany and the Netherlands (Rootsi et al., 2004).

<table>
<thead>
<tr>
<th></th>
<th>Sk6866</th>
<th>Sk6990</th>
<th>Sk6994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>15-19 years</td>
<td>Middle Adult</td>
<td>Young Adult</td>
</tr>
<tr>
<td>Osteological sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Molecular sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Sampled material</td>
<td>second premolar</td>
<td>second incisor</td>
<td>second incisor</td>
</tr>
<tr>
<td>Genome coverage</td>
<td>0.04</td>
<td>0.19</td>
<td>0.83</td>
</tr>
<tr>
<td>mtDNA genome</td>
<td>260.8</td>
<td>366.6</td>
<td>379.8</td>
</tr>
<tr>
<td>SNPs overlapping HO db</td>
<td>16,550</td>
<td>83,210</td>
<td>242,251</td>
</tr>
<tr>
<td>SNPs overlapping EGDP db</td>
<td>1,476,314</td>
<td>6,907,830</td>
<td>20,272,957</td>
</tr>
<tr>
<td>mtDNA haplogroup</td>
<td>U5b2a</td>
<td>T2b21b</td>
<td>I1a1b</td>
</tr>
<tr>
<td>Y chr haplogroup (ISOOG)</td>
<td>R1b1a</td>
<td>R1b1</td>
<td>I2a2a</td>
</tr>
<tr>
<td>Contamination estimate</td>
<td>1.6% (0.85%-2.36%)</td>
<td>2.08% (1.54%-2.62%)</td>
<td>1.29% (0.81%-1.78%)</td>
</tr>
</tbody>
</table>

Table 1. Summary statistics of the genomic and anthropological data from the three analyzed individuals.

The $f_3$-outgroup statistics were performed with two modern population reference panels: Human Origins (Lazaridis et al., 2014; Patterson et al., 2012) merged with seven 17th century Swedish genomes and Estonian Biocentre Human Genome Diversity Panel (EGDP) (Pagani et al., 2016) using Yoruban population as an outgroup and 51 populations from the Human Origin reference panel and Congo Pygmy population as an outgroup with 125 populations from EGDP. The number of SNPs used in the $f_3$-outgroup statistics was 68250 and in individual Sk6994, 23229 and 6907830 in individual Sk6990 and 4768 and 1476314 in individual Sk6866 for HO+ancient and EGDP reference panels respectively. The standard errors were obtained by performing a block jackknife with the number of blocks ranging from 557 to 699. According to the $f_3$-outgroup test with HO+ancient, individual Sk6866 shared most genetic drift with the Baltic and central European populations (i.e. Belarusian, Lithuanian, Estonian, Norwegian and ancient Swedish).
Individual Sk6990 shared most genetic drift with the Baltic and western European populations (including Lithuanian, English, Basque, Czech, French and ancient Swedish). And individual Sk6994 shared most genetic drift with the western and northern European populations (including Lithuanians, English, Orcadians and Norwegians) (Figure 2). When compared to the $f_3$-outgroup test with EGDP Sk6866 shared most genetic drift with Swedes, Western Russians and Poles; Sk6990 shared most genetic drift with Swedes and Belarussians, then Finnish, while Sk6994 shared the most genetic drift with present-day Lithuanians, Swedish, and Latvians (Figure 3).

The $D$-statistics were performed to test whether investigated individuals shared excess drift with present day Scandinavians, here represented by Norwegians/Swedish/ancient Swedish, or individuals from other European geographical regions with either Yoruban or Congo Pygmies population as an outgroup depending on the reference panel used. A selection of $D$-statistic results is presented in Table 2. As expected most $D$-statistic results had low values, oscillating around 0, but a number of significant Z score values ($Z>2$) suggest that individual Sk6866 shared more genetic drift with Norwegians and Swedish than southern and western European populations, such as the French, the Italians and the Germans. Individual Sk6990 had broad European affiliations sharing most affinities with the Swedish, Lithuanians and Norwegians. Finally, individual Sk6994, shared most genetic drift with the Swedish and Norwegians (Table 2).

<table>
<thead>
<tr>
<th>Reference population (DB)</th>
<th>Individual 6866</th>
<th>Individual 6990</th>
<th>Individual 6994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgian (HO)</td>
<td>0.0077</td>
<td>-0.003</td>
<td>-0.0164</td>
</tr>
<tr>
<td>Czech (HO)</td>
<td>-0.0083</td>
<td>0.0058</td>
<td>-0.0076</td>
</tr>
<tr>
<td>Estonian (HO)</td>
<td>0.0012</td>
<td>-0.0035</td>
<td>-0.0054</td>
</tr>
<tr>
<td>Lithuanian (HO)</td>
<td>0.0046</td>
<td>0.0092</td>
<td>0.0024</td>
</tr>
<tr>
<td>FinnishFIN (HO)</td>
<td>-0.0055</td>
<td>0.0013</td>
<td>-0.0097</td>
</tr>
<tr>
<td>French (HO)</td>
<td>-0.0185</td>
<td>0.0014</td>
<td>0.0076</td>
</tr>
<tr>
<td>GreekComas (HO)</td>
<td>-0.0144</td>
<td>-0.0093</td>
<td>0.018</td>
</tr>
<tr>
<td>Population</td>
<td>D-statistic Test Results</td>
<td>p-value</td>
<td>q-value</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Italian Tuscan (HO)</td>
<td>-0.0389</td>
<td>3.116*</td>
<td>0.0018</td>
</tr>
<tr>
<td>Ancient Swedish (HO)</td>
<td>-0.0039</td>
<td>0.218</td>
<td>0.0021</td>
</tr>
<tr>
<td>Belarusians (EGDP)</td>
<td>-0.0032</td>
<td>0.615</td>
<td>-0.0014</td>
</tr>
<tr>
<td>Poles (EGDP)</td>
<td>-0.0015</td>
<td>0.268</td>
<td>-0.0044</td>
</tr>
<tr>
<td>Germans (EGDP)</td>
<td>-0.0133</td>
<td>2.361*</td>
<td>-0.008</td>
</tr>
<tr>
<td>Estonians (EGDP)</td>
<td>-0.0044</td>
<td>0.893</td>
<td>-0.014</td>
</tr>
<tr>
<td>Lithuanian (EGDP)</td>
<td>-0.009</td>
<td>1.607</td>
<td>-0.0024</td>
</tr>
<tr>
<td>Karelians (EGDP)</td>
<td>-0.0081</td>
<td>1.405</td>
<td>0.0166</td>
</tr>
<tr>
<td>Finnish (EGDP)</td>
<td>-0.0105</td>
<td>1.869</td>
<td>-0.0016</td>
</tr>
<tr>
<td>Ukrainians West (EGDP)</td>
<td>-0.016</td>
<td>2.805*</td>
<td>-0.009</td>
</tr>
<tr>
<td>Western Russian (EGDP)</td>
<td>0</td>
<td>0.007</td>
<td>-0.0023</td>
</tr>
</tbody>
</table>

Table 2. Selection of the D-statistic test results. Depending on the reference panel used (either HO or EGDP) the population tree topology tested was: (Yoruba/Congo Pygmies, sample: Norwegian/Swedish, reference population), where either ‘Yoruba’ or ‘Congo Pygmies’ are the outgroup, ‘sample’ is any of the ancient individuals and ‘reference population’ is comparative population from the Human Origins dataset merged with ancient Swedish individuals.

DISCUSSION

The samples in the present study are approximately 400 years old. Out of the original seven sampled individuals, four yielded DNA, and in three of those the proportion of human DNA was >1%, (which is a cut-off value we employ to decide whether further, non-enriched, shotgun sequencing is feasible). The three individuals were thus sequenced further to obtain suitable amount of genomic data for downstream analyses. The proportion of human DNA in a sample is generally a good indicator of endogenous DNA preservation, as long as presence of major contaminants can be excluded. Here, mitochondrial DNA based testing indicated that the three samples were relatively free from contamination, and the damage patterns suggest that much of the DNA in these samples is of ancient origin.

On PCA plots Sk6866 clusters between Ukrainians and Scandinavians (Figure 2A, 3A). At the
same time the $f_3$-outgroup statistics provides a somewhat stronger support for shared drift with Swedes and populations on the eastern side of the Baltic Sea (Western Russians). The $D$-statistics suggests that this individual shared most genetic with populations from the north, especially Swedish. The mitochondrial haplogroup is one that is common among modern Finns, Saami, Estonians, and Ukrainians (Malyarchuk et al., 2010). Thus, this individual is likely from Sweden or the eastern region of the Baltic Sea (Figure 2B, 3B).

Sk6990 clusters with central and northern European populations on the PCA plot, and this is also where the $D$-statistics indicate he has his affinities, since shared drift with some southern European populations can be excluded and most genetic drift is shared with the Swedish. The mitochondrial haplogroup is one that is widespread in Europe (Pala et al., 2017), as is the Y haplogroup (Myres et al., 2011). However, the $f_3$-outgroup statistics points to the north and shared genetic drift with the Swedish, Finnish and Baltic populations including Western Russian (Figure 2C, 3D).
Figure 2. A) Principle Component Analyses visualizing the relation between the three investigated individuals from Sala silver mine and seven ancient Swedish individuals compared to a panel of reference of 51 modern populations form Human Origins dataset. B-D) Outgroup $f_{st}$-statistics calculated between each of the ancient individuals and modern populations from Human Origins reference panel (Patterson et al., 2012; Lazaridis et al., 2014). The comparative ancient population is marked by a star.
Figure 3. A) Principle Component Analyses visualizing the relation between the three investigated individuals from Sala silver mine and seven ancient Swedish individuals compared to a panel of reference of 13 modern populations from Estonian Biocentre Human Genome Diversity Panel (EGDP) (Pagani et al 2016). B-D) The 15 highest $f_3$-statistics values calculated between each of the ancient individuals and 125 modern populations Estonian Biocentre Human Genome Diversity Panel (EGDP) (Pagani et al 2016). *(NB! The Figure is still undergoing editing to make it more aesthetically pleasing)*

Sk6994 also clusters with central and northern European populations on the PCA plot, similarly to Sk6990. The $f_3$-outgroup statistics shows affinities for the same area as do individuals with a historic background in northern Europe (Rootsi et al., 2004) albeit with most shared genetic drift with the present-day Swedish, Lithuanians and Latvians. This is also supported by the $D$-statistics suggesting shared drift with a modern population from these areas, more specifically with Scandinavia, Norway and Sweden. The mitochondrial haplogroup is one that is common in Denmark and southern and central Scandinavia, but also in many other parts of Europe (Figure
CONCLUSIONS

The three individuals reported in this study genetically represent a group of north Europeans with close affinities to contemporary and modern Swedish. Both PCA plot and D-statistic analyses suggest that these individuals are not German, Polish nor Russian. The two latter being countries at war with Sweden at that time so a potential source of war prisoners. These results narrowed down the probable origin of the individuals analyzed. However, due to the absence of modern and contemporary Danish genomic data we could not refute hypothesis that these individual were war prisoners from Denmark. This study highlights the difficulties of fine scale differentiation in historical times between European neighboring countries with a recent common ancestry and an intermittent shared history. On the other hand close affinities to present-day Swedish and the fact that tested individuals fall within genetic variation of contemporary Swedish combined with strontium results suggest that these individuals likely were from southern Sweden. It is noteworthy that the double burial from which two individuals (Sk6990 and Sk6994) (Figure 1b) were sampled expressed little homogeneity. Different strontium values suggested that the two men grew up in different areas (Bäckström and Price, 2016). Moreover, the men did not share neither mitochondrial nor Y chromosome lineage thus they were not members of a nuclear family unit. The finding is in line with expectation that the burial ground was populated by aliens who may have been foreign soldiers or Swedish convicts since such individuals are not expected to be close relatives. That is in sharp contrast to what is known about silver mining in Sala, where free workers are believed to have worked in pairs consisting of related males (Tallqvist, 2007). However, taken together the genetic and isotopic results are consistent with the three individuals originating in southern Sweden.
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