1   **Analysis of ancient human mitochondrial DNA from Verteba Cave, Ukraine: insights into**

2   **the origins and expansions of the Late Neolithic-Chalcolithic Cututeni-Tripolye Culture**

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4   Ken Wakabayashi[1], Ryan W. Schmidt*[1,2], Takashi Gakuhari[1,3], Kae Koganebuchi[1], Motoyuki

5   Ogawa[1], Jordan K. Karsten[4], Mykhailo Sokhatsky[5] and Hiroki Oota*[1]

6

7   [1]Department of Anatomy, Kitasato University, 1-15-1 Kitsasato, Sagamihara, Kanagawa

8   252-0374, Japan

9   [2]School of Archaeology, Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland

10   [3]Kanazawa University, Center for Cultural Resource Studies, Kakuma, Kanazawa, Ishikawa

11   920-1164, Japan

12   [4]Department of Anthropology and Religious Studies, University of Wisconsin-Oshkosh,

13   Oshkosh, WI 54901

14   [5]Borschiv Regional Museum, Ministry of Culture and Arts, Shevchenka St. 9, Bilche Zolote,

15   Ukraine

16

17   *Correspondence: Ryan W. Schmidt, Ph.D., and Hiroki Oota, Ph.D.

18   (E-mail: schmidtrw@gmail.com ; hiroki_oota@med.kitasato-u.ac.jp)

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1   **Abstract**

2   **Background:** The Eneolithic (~ 5,500 yrBP) site of Verteba Cave in Western Ukraine contains

3   the largest collection of human skeletal remains associated with the archaeological

4   Cucuteni-Tripolye Culture. Their subsistence economy is based largely on agro-pastoralism and

5   had some of the largest and most dense settlement sites during the Middle Neolithic in all of

6   Europe. To help understand the evolutionary history of the Tripolye people, we performed

7   mtDNA analyses on ancient human remains excavated from several chambers within the cave.

8   **Results:** Burials at Verteba Cave are largely commingled and secondary in nature. A total of 68

9   individual bone specimens were analyzed. Most of these specimens were found in association

10   with well-defined Tripolye artifacts. We determined 28 mtDNA D-Loop (368 bp) sequences

11   and defined 8 sequence types, belonging to haplogroups H, HV, W, K, and T. These results do

12   not suggest continuity with local pre-Eneolithic peoples, but rather complete population

13   replacement. We constructed maximum parsimonious networks from the data and generated

14   population genetic statistics. Nucleotide diversity (π) is low among all sequence types and our

15   network analysis indicates highly similar mtDNA sequence types for samples in chamber G3.

16   Using different sample sizes due to the uncertainly in number of individuals (11, 28, or 15), we

17   found Tajima's D statistic to vary. When all sequence types are included (11 or 28), we do not

18   find a trend for demographic expansion (negative but not significantly different from zero);

19   however, when only samples from Site 7 (peak occupation) are included, we find a significantly

20   negative value, indicative of demographic expansion.

21   **Conclusions:**   Our results suggest individuals buried at Verteba Cave had overall low mtDNA

22   diversity, most likely due to increased conflict among sedentary farmers and nomadic

23   pastoralists to the East and North. Early Farmers tend to show demographic expansion. We find

24   different signatures of demographic expansion for the Tripolye people that may be caused by

25   existing population structure or the spatiotemporal nature of ancient data. Regardless, peoples of

26   the Tripolye Culture are more closely related to early European farmers and lack genetic

27   continuity with Mesolithic hunter-gatherers or pre-Eneolithic groups in Ukraine.

28   **Keywords:**   mtDNA, ancient DNA, Tajima's D, Tripolye, Eneolithic, Ukraine

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1   **Background**

2   Profound cultural transitions accompanied the Neolithic and Bronze Age in Europe.

3   These transitions were catalyzed initially through large-scale migrations of agriculturists from

4   the West Asia (modern day Anatolia) during the Neolithic, and later through the migrations of

5   steppe herders from the Pontic-Caspian and Volga regions of modern Ukraine during the

6   Bronze Age [1-4].   A continuing question of debate among researchers is how these migrations

7   affected the genetic composition of modern-day Europe [5, 6].   A key question in this debate is

8   whether these migrations that brought about cultural changes were the results of movements of

9   people (demic diffusion model), as opposed to the movement of ideas and artifacts (cultural

10   diffusion model) [7].   That is, were these large-scale migrations a process of cultural diffusion,

11   with little genetic admixture among early Neolithic farmers and Mesolithic hunter-gatherers?

12   Or, is a model of demic diffusion [7-10] more appropriate, whereby different regions of Europe

13   were more or less affected by admixture with early farmers, and later steppe herders during the

14   Bronze Age?

15   The transition to farming from a foraging lifestyle first appeared in the Near East around

16   10,500 years before present (yrBP) in modern-day southeastern Anatolia and Syria.

17   Archaeologists have described two major and contemporaneous routes of expansion, namely the

18   Continental (Danubian) and Mediterranean route.   By 9,500 yrBP, farming spread into parts of

19   Central Europe through the migration of peoples associated with the Linear Pottery culture (or

20   *Linearbandkeramik*, LBK).   These LBK cultures originated in Hungary and Slovakia (the

21   Carpathian Basin) and then spread rapidly as far as the Paris Basin and Ukraine.   A lingering

22   question among archaeologists has always been whether these first farmers were descendants of

23   local hunter-gatherers or whether they migrated from the Near East.   Paleogenetic studies have

24   generally suggested these early farmers were migrants, though in some places peoples continued

25   to admix after the adoption of agriculture [5, 11-14.]

26   The region of Southeastern Europe (SE) has not been as extensively investigated as

27   Southwestern (SW) Europe, Central Europe, or Southern Scandinavia, in terms of their ancient

28   DNA variation.   In contrast to Central Europe, the area of what is modern Ukraine saw the

29   adoption of agriculture late.   Although features of the Neolithic package are visible in Ukraine

30   as early as 8,500–7,500 years before present (we use calBP to indicate radiocarbon dating),

31   agriculture was not adopted as a primary subsistence economy until the Eneolithic or

32   Chalcolithic period (around 5,500 yrBP) [15].   Whereas Central Europe saw mostly the

33   process of demic diffusion [8], SE Europe seems to have adopted agriculture through innovative

1   subsistence strategies through a transfer of ideas, little genetic influence, and genetic continuity

2   from the Mesolithic to the Neolithic.   Therefore, the Neolithic transition occurred at a slower

3   pace, thus perhaps shaping the genetic composition of this region differently than in other parts

4   of Europe.

5   Following the establishment of farming communities in the Balkan Peninsula, a series of

6   complex societies formed, culminating in large settlements.   By 5,500 yrBP, agriculture had

7   reached Eastern Europe, in the form of the Cucuteni-Trypillian (C-T) complex in the area of

8   present day Moldova, Romania, and Ukraine.   This culture spanned close to 2,000 years and

9   influenced much of SE Europe and the Baltic regions.   It is known for elaborate

10   anthropomorphic and animal figurines, as well as distinct elegantly painted pottery.   Around

11   4,000 yrBP, these societies began to change, with the large settlements being abandoned, and

12   archaeological evidence suggesting contact with nomadic steppe populations from the East.

13   We address the complex process of Neolithisation [7] in SE Europe by examining an

14   Eneotlithic (Chalcolithic) Cucuteni-Trypillian site from Ukraine.   For brevity, we will use the

15   term Tripolye, as this is what it is known in Ukraine.   Tripolye culture (7,100 – 5,000 calBP) is

16   defined as Eneolithic based on the presence of copper artifacts and the onset of metallurgy, and

17   ends at the beginning of the Bronze Age.   The Tripolye culture occupied a large area from the

18   Carpathian Mountains in the west to the Dnieper River in the east, and extended as far south as

19   the Black Sea and north to Kiev (Fig. 1).   Relative and absolute chronologies divide the

20   Tripolye Culture into several phases (see Additional file 1), which normally accompany

21   changes in pottery manufacture and decoration [16]. People associated with this culture are

22   known as Trypillians.

23   In the present study, we investigate human remains found at a single Tripolye site known

24   as Verteba Cave (VC).   The occupation of the cave spanned over 1000 years, making it ideal to

25   investigate the origins and possible genetic changes through time in the Trypillian people.

26   Verteba Cave has been excavated as an archaeological site since the 1820s, though more

27   intensive excavation has been ongoing since 1996 under the direction of co-author M.

28   Sokhatsky (Borschiv Regional Museum of the Ukrainian Ministry of Culture and Arts).

29   Verteba is a gypsum cave located in Western Ukraine, in the boreal forest-steppe zone of the

30   East European Plain.   It is one of many vast underground cave systems in the region.

31   Interpretations surrounding the use of the cave vary during these periods.   Some believe

32   the cave was used as a temporary shelter, while increasing archaeological evidence suggests use

33   as a ritual site or a mortuary function.   There is also evidence to support the idea that

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1   individuals buried in the cave, which are largely secondary in nature, are victims of warfare or

2   sacrifice, due to the high frequency of blunt force trauma [17, 40]. Regardless, the cave contains

3   the largest accumulation of human remains associated with the Tripolye culture.   In fact, very

4   few Tripolye culture human remains exist, making the cave one of the most important sites to

5   investigate diet, health, pathology, and population history of Trypillian peoples [18, 19].

6   Using data obtained from the mitochondrial HVR-I region, we ask several interrelated

7   questions about individuals buried at Verteba Cave.   First, is there evidence for a maternal

8   genetic continuity, and thus a degree of cultural diffusion, with local Mesolithic

9   hunter-gatherers, as suggested in several recent studies?   If there is not evidence for this, how

10   are these individuals related to earlier farmer groups from SE or Central Europe? Is there any

11   indication of a steppe influence for the Trypillian peoples?   And lastly, can we infer something

12   about Trypillian people's expansion or collapse using maternal lineages?   Several studies

13   [20-22] have briefly addressed two of these three questions, indicating a link to early Neolithic

14   farmers with some possibility for a link with Mesolithic hunter-gatherers.   However, in those

15   studies, the sample sizes tended to be smaller and the human remains used came from only a

16   single chamber. Here, we analyze mtDNA data from several chambers in different locations

17   found throughout the cave in an attempt to answer these questions.

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20   **Results**

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22   To test reproducibility, we cut each specimen into 2 or 3 pieces and extracted DNA from

23   each, and obtained 156 DNA extractions from 63 specimens (53 bones and 10 teeth) from

24   chambers in the VC cave (Fig. 1).   Using the DNA extractions as templates, we conducted

25   PCR-amplification, and successfully amplified 38 specimens using all three defined overlapping

26   primer-sets (Table 1).   We sequenced 114 PCR amplicons using the Sanger method on an ABI

27   3130 Genetic Analyzer, and obtained sequences from 34 specimens for all three amplicons.

28   We removed sequences from 6 specimens with amplicons that were mutually incompatible in

29   nucleotide sequences, and finally obtained highly reliable 368-bp sequences from 28 specimens

30   (Table 2, Additional file 2)

31   Eight defined sequence types ('Seq Type') of mtDNA D-loop were detected in 28

32   specimens, as well as the sequence types for two of us (K.W. and R.S.), who worked on DNA

33   extraction. K.W. and R.S. sequence types were not identical to the eight sequence types except

1   for Seq Type II from one bone specimen that was identical to the sequence type of R.S. (Table 2,

2   Additional file 3).   This sequence type corresponds to mitochondrial haplogroup HV12b,

3   which is common in modern European populations.   We could not eliminate the possibility

4   that it is contamination, but included it in subsequent analyses.   In addition to Seq type II,

5   sequence type VI was predicted to be haplogroup HV.   Sequence types I, III, and IV were

6   predicted to be haplogroup H, while sequence types V, VII, and VIII were predicted to be

7   haplogroups W, K, and T, respectively.   Thus, all the haplotypes found in the VC specimens

8   are common in modern European populations.

9   Based on the 8 sequence types of the mtDNA D-loop, a maximum parsimonious

10 phylogenetic network was constructed (Fig. 2).   Circles represent the sequence types, and the

11 size of the circle is proportional to the number of samples.   Numbers on the branches between

12 the circles are nucleotide position numbers (+16,000) of the human mitochondrial genome

13 sequence (rCRS [23]).   Information about the location (chamber within the cave) where the

14 specimen was excavated is also provided.   Areas 2 and 17 are part of Site 7, and these are

15 defined as a separate chamber, although they are located in close proximity within Site 7.   The

16 other chambers, Site 20, G2, and G3, are independent and separate locations within the cave.

17 'Undefined' chamber describes an unknown location within the cave.

18   Specimens from each chamber showed deviation for the sequence type distribution

19 observed in the sample set (Fig. 2).   For example, specimens excavated from Site 7 had five

20 unique sequence types, (I, II, III, IV, and VIII), while specimens excavated from chamber G3

21 had mainly one sequence type (V).   For those specimens with the same sequence type, there

22 are two possibilities: (1) the specimens belong to the same maternal lineage through several

23 generations, or (2) the specimens (bone fragments and/or teeth) are from the same individual.

24 This could be the case as all of the elements from site G3 were different (Size 28 in Tables 3, 4,

25 5).   However, because archaeological excavation has not been completed for site G3, it is

26 unclear which of these two possibilities is the case.   We consider the two possibilities as both

27 plausible.   If the first possibility is correct, then we have 28 distinct individuals.   If these

28 lineages all come from the same individual, then we are left with only 11 individuals (Size 11 in

29 Tables 3, 4, 5).

30   We next calculated population genetic statistics for the VC specimens (conducting analyses

31 for both 28 and 11 individuals separately) and made comparisons with modern populations

32 (Table 3).   If we consider all the specimens belonging to a single population, then the number

33 of sequence types, 8, was quite small compared to modern populations.   Nucleotide diversity

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1 (π) in VC was 0.00634 when using 28 specimens as a population, and was 0.00919 when we

2 only consider the total sample size to be 11.   Both values of π are smaller than those of many

3 modern populations, and relatively similar to those of Basques (0.00894), Norway (0.00969),

4 Sweden (0.00909), and Switzerland (0.00971).

5 Tajima's D statistic is a test of neutrality for nucleotide sequences, but when testing an

6 obviously neutral locus (mtDNA), it suggests something about a population's demographic

7 history [24].   If Tajima's D is statistically significant negative, then it suggests that the

8 population has experienced demographic expansion.   If Tajima's D is not significantly

9 different from zero, then it suggests that the population has maintained a constant population

10 size [25].   Tajima's D values of VC specimens were negative but not significantly different

11 from zero.   Thus, population genetic statistics of the VC specimens suggest that, if they are a

12 contemporaneous population, then their genetic diversity was as small as modern Basques and

13 Scandinavians who likely experienced bottleneck effects, and based on Tajima's D test, the VC

14 people were unlikely to be a population that experienced demographic expansion.

15 We compared the frequencies of the haplogroups predicted based on mtDNA D-loop

16 sequences in the VC with those in the modern and the ancient populations from Europe and

17 Asia (Table 4, 5).   Haplogroup H was the most frequent (45.5 ~ 57.1%), and haplogroups W

18 and HV* were the second most frequent (9.1 ~ 25.0% and 7.1 ~ 18.2%, respectively) in our

19 samples.   Haplogroup H is among the most common for modern Western, Central, and Eastern

20 European populations, but the haplogroups W and HV* are relatively less frequent in modern

21 European populations [26].   On the other hand, haplogroup H is less frequent in Central and

22 West Asia, but slightly higher frequencies of haplogroup HV* are reported for those regions.

23 In individuals from VC, haplogroups T and K also showed significant frequencies (3.6 ~ 9.1%

24 and 7.1 ~ 18.2%, respectively).   The ranges of the frequency of haplogroups reported here fall

25 within the variation of modern European and West Asian populations (Table 4).

26 In comparison with ancient groups, VC haplogroup frequency is most similar to Neolithic

27 farmer groups from Central Europe, as well as Funnel Beaker groups from Central (Salzmünde,

28 Baalberge Cultures from Germany) and Northern Europe, particularly with respect to

29 haplogroups H, HV, and T (Table 5). The Funnel Beaker cultural complex overlapped in

30 territory with the Tripolye culture in the upper parts of the Dniester River and adjacent areas,

31 and there is evidence for archaeological contact between the two cultures [21].

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Discussion

In this study we have attempted to answer a number of related questions surrounding Trypillian population history using data gleaned from maternal ancestry. We include at most 28 and a minimum of 11 individuals buried at Verteba Cave, an Eneolithic site associated with the Tripolye culture. Though we understand the limitation in using such data, we suspect our findings answer (in part), some fundamental aspects of Trypillian people's genetic affiliation with other Neolithic groups.

To address the complexities associated with the transition to farming (or the process of Neolithisation), a wealth of ancient mtDNA sequences has been amassed from the Late Mesolithic to the Late Bronze Age [5, 27]. To explain these genetic changes in various regions of Europe, it is important to fully understand the genetic substratum spanning the Mesolithic-Neolithic transition. Studies have suggested that the maternal signature of local hunter-gatherer groups in many parts of Europe was homogenous with a relatively small population size and haplogroups dominated by lineage haplogroup U, such as U2, U4, U5a, U5b, and U8. Conversely, the genetic lineages of Early Neolithic farmers from Central and Southwest Europe are compromised by a wider array and diversity, with U lineages being less frequent. The mitochondrial Neolithic package arrived in Central Europe around 8,000 years BP with cultures associated with LBK farmers and the lineages are largely assigned to haplogroups N1a, T2, K, J, HV, V, W, and H. These lineages largely replaced local signatures of peoples associated with Mesolithic cultures, at least for areas of Central Europe [5].

We first explored whether individuals buried at Verteba Cave are more closely related to earlier Neolithic farmers from Central Europe, or perhaps have some connection with local hunter-gatherers, thus emphasizing the role of cultural diffusion in the adoption of agriculture. mtDNA haplogroup data for modern and ancient populations in Europe and West Asia have shown there was a discontinuity between late hunter-gatherers and early farmers, and later extant European populations, in most locations throughout Europe. An exception has been in SE Europe and the Baltic where cultural diffusion may have played a larger role [28].

The VC haplotype distribution indicates common haplotypes among Eurasian populations [26]. These include maternal haplogroups H, T, K, and W. The majority of haplotypes fall into haplogroup H, which is the most common haplogroup among modern-day Europeans and peoples of the West Asia, accounting for around 40% in Europeans [29] and is found in approximately 44% of modern Ukrainians [30]. This suggests that population continuity

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1   between the Tripolye and modern Ukrainians is a distinct possibility.   Further, one individual

2   displayed haplogroup T2b (Table 1).   In addition to T2b being a possible marker of Anatolian

3   expansion [5], it has also been found at high frequency in the Carpathian Mountains [31].   In

4   one study [31], an individual from Bilche Zolote was found to have haplogroup T2b.   Bilche

5   Zolote is only 3 kilometers from the site of Verteba Cave, indicating some degree of local

6   continuity with the Trypillian people.

7   Previous ancient DNA studies showed that hunter-gatherers before 6,500 yrBP in Europe

8   commonly had haplogroups U, U4, U5, and H, whereas hunter-gatherers after 6,500 yrBP in

9   Europe had less frequency of haplogroup H than before [5].   Haplogroups T and K appeared in

10   hunter-gatherers only after 6,500 yrBP, indicating a degree of admixture in some places

11   between farmers and hunter-gatherers.   Farmers before and after 6,500 yrBP in Europe had

12   haplogroups W, HV*, H, T, K, and these are also found in individuals buried at Verteba Cave

13   (Table 5) [32]. Therefore, our data point to a common ancestry with early European farmers.

14   Our data also suggest population replacement.   Mathieson et al. [42] analyzed a number of

15   Neolithic Ukrainian samples (petrous bone) from several sites in southern, northern, and

16   western Ukraine, dating to ~8,500 – 6,000 yrBP, and found exclusively U (U4 and U5) mtDNA

17   lineages.   It should be noted that 'Neolithic' in this context does not mean the adoption of

18   agriculture, but rather simply coinciding with a change in material culture.   They also analyzed

19   several Trypillian individuals from Verteba Cave (different samples from the those included in

20   this study).   Similar to our findings, they found a wider diversity of mtDNA lineages,

21   including H, HV, and T2b.   These data, combined with our results, appear to confirm almost

22   complete population replacement by individuals associated with the Tripolye Culture during the

23   Middle to Late Neolithic.

24   Although we did not find any haplotypes common among hunter-gatherers (U lineage),

25   there exists the possibility that some Trypillians inherited this haplotype from Mesolithic

26   hunter-gatherers and thus some degree of cultural diffusion.   In a study by Nikitin et al. [21] on

27   mtDNA variation at Verteba Cave using teeth and cranial fragments, they found haplogroup

28   composition similar to our results (mainly haplogroups H, HV, and T).   However, Nikitin et al.

29   [21] also found two individuals to have haplogroups U8 (U8b1a2 and U8b1b), which have been

30   found among Paleolithic specimens [33].   Haplotype U8b1 has also been discovered in

31   Neolithic Anatolian farmers [4].   Based on the data in [21], they were unable to distinguish

32   whether their individuals with U8 were more similar to Paleolithic or West Asians from the

33   Neolithic.   Based on these data combined with our preliminary results, it appears the

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1   Trypillians were very much a distinct people who most likely displaced local hunter-gatherers

2   with little admixture.

3   Haplogroup W was also observed in several specimens deriving from Site G3.   Although

4   we are unsure if all of these haplogroups come from a single or multiple individuals, this

5   observation is interesting in that it is relatively rare and isolated among Neolithic samples.   It

6   has, however, been found in samples dating to the Bronze Age (Table 5) [35].   In the study by

7   Wilde et al. [35], they found haplogroup W present in two samples from the Early Bronze Age

8   associated with the Yamnaya and Usatovo cultures.   The Usatovo culture (~ 3500 – 2500 BC)

9   was found in Romania, Moldova, and southern Ukraine.   It was the conglomeration of

10   Tripolye and North Pontic steppe cultures.   Therefore, this individual could link the Trypillian

11   peoples to the Usatovo peoples and perhaps to the greater Yamnaya steppe migrations during

12   the Bronze Age that lead to the Corded Ware Culture [36].

13   Verteba Cave contains archaeological evidence for the Tripolye cultural complex,

14   including implements for agrarian cultivation, including grain processing.   Based on the

15   material culture, the immensity of certain settlements (some housing up to 10,000 people [34]),

16   as well as the deterioration in biological health resulting from grain consumption, it is clear the

17   subsistence economy of the Trypillians was reliant on agriculture.   Population genetic statistics,

18   in the form of Tajima's D test, based on all VC mtDNA sequence data show that the Trypillian

19   people (at least their maternal lineages) did not experience demographic expansion, at least

20   during the Late Neolithic.   Previous studies report that modern hunter-gatherers do not indicate

21   a signal of demographic expansion in mismatch distribution and/or Tajima's D test but farmers

22   tend to show expansion based on increasing numbers of individuals living in sedentary

23   conditions [37, 38].

24   Although we find a negative value for Tajima's D when analyzing all of the sampled

25   individuals (n=28), it is not significant.   However, we are unable to treat our sample population

26   as being contemporaneous.   Although most individuals included in this study come from Site 7,

27   which has been firmly established to date around 5,500 cal BP based on ceramics and

28   radiocarbon dating [20, 39], other chambers in the cave are not as confidently dated.   For

29   example, chamber G3 had very few artifacts that came from the Tripolye culture.   Given this

30   caveat, we analyzed only individuals buried in Site 7 (n = 15) and found that Tajima's D is

31   negative (-1.50475) and significant (p = 0.04900).   We theorize that this is due to the fact that

32   individuals buried at Site 7 come from the time of peak occupation in the cave, and therefore

33   were experiencing population aggregation and increased density, and therefore show a signal of

1   demographic expansion. However, when we include individuals from other chambers (such as

2   G3 that most likely date to a later period), we could be seeing the effects of population structure,

3   which might cause the negative, but non-significantly different from zero of Tajima's D

4   indicating population size stability.

5   An alternative explanation for our observed population size stability when all individuals

6   are included might be due to sampling strategy and the temporal component of ancient DNA

7   sites. If a population migrates in low numbers into a new environment, such as the case with

8   early migrating farmers from Anatolia, we would slowly see an increase in the population as

9   they become increasingly sedentary over time. If we were to sample from this site and test for

10   demographic expansion, we would most likely see that reflected in a statistic like Tajima's D.

11   As the population increases and resources reach an upper limit, groups would begin to fission

12   and settle into new locations in close geographic proximity. Sampling individuals from each

13   of these new sites, we would expect to see the maintenance of population stability since the

14   groups, though genetically related, are spread out and thus would maintain population

15   equilibrium with bi-directional migration. If, at some points in time, these sites again become

16   aggregated because of increased population size, and we were to sample from this new, larger

17   archaeological site, we would again witness demographic expansion simply because the

18   population size has increased on the whole. Therefore, farmer populations as a whole (over

19   the course of the Neolithic), generally see a trend for increased population expansion as local

20   villages turn into larger settlements (as could be the case for peak occupation at Verteba Cave,

21   where nearby settlements tended to be large). However, if we sample from each of those

22   localities over time, as perhaps we are seeing with our results when we include samples from all

23   sites (chambers), then we do not see demographic expansion, but rather maintenance of

24   population size over time.

25   Archaeologically, it has been documented that Tripolye settlements began to disappear at

26   the beginning of the Bronze Age. The reasons for this vary, but could be influenced by their

27   interaction with steppe groups from the east. One of the possibilities for settlement

28   abandonment is warfare. It has been well documented at Verteba that inter-personal violence

29   was a common phenomena [17, 41]. Madden et al. [41] found a high degree of trauma-related

30   cranial injuries among Trypillian burials. It is believed these individuals were killed by a

31   raiding outside group and were later buried by members of the Tripolye culture. The

32   Trypillians were one of the last of the "Old Europe" cultures that lived along the shores of the

33   Danube River. By 5,800 calBP, many of these Neolithic Danubian cultures were wiped out after

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1   the arrival of pastoralists from the steppe [17].   It is believed that by 5,300 calBP, the

2   Trypillians were in conflict with members of the Usatovo culture to the south, no longer

3   benefitting from trade relationships across the forest-steppe boundaries.   A reduction in

4   mtDNA diversity, as shown by our results, could be explained through the targeted killing of

5   both male and female Trypillians.

6   Another explanation for the sudden collapse of Tripolye culture, the crisis in Neolithic

7   societies, and the observation of non-demographic expansion may be due to recent

8   documentation of an early form of plague that was widespread from Siberia to the Baltic around

9   5,000 years BP [41].   Neolithic communities contracting this early form of the plague would

10 have devastated Tripolye mega-sites, thus creating a demographic collapse that we are only

11 glimpsing in our population genetic analyses.   To get a better understanding of the

12 demographic collapse in Tripolye society, we will need to obtain genome-wide data to further

13 explore how these early agro-pastoralists eventually declined or were replaced by steppe

14 nomads from the East.

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16 **Materials and Methods**

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18 **Samples**

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20 Human remains for DNA analyses come from several excavation sites located within Verteba

21 Cave (VC).   VC is a mortuary site located outside the modern village of Bilche Zolote,

22 Ternopil Oblast, Ukraine (Fig. 1).   Most samples date to the Tripolye CII period (~5,500 BP)

23 based exclusively on associated pottery found in the same cultural layer as the human remains

24 (Additional file 1).   Nikitin et al. [21] radiocarbon dated human and animal remains, as well as

25 pottery sherds from Verteba and found the dates correspond to transitional phases in pottery

26 decoration, with peak activity placed around 5,500 calBP.   Almost all skeletal samples

27 excavated within Verteba Cave are commingled and individual burials are difficult to identify.

28 Therefore, in order to avoid sequencing the same individual twice, one of us (J.K.) collected

29 second right metacarpal bones for analysis from a single chamber (Site 7).   These samples

30 were collected over several excavation field seasons (2008-2014).   The remains were initially

31 kept at the University of Wisconsin-Oshkosh, and later transferred to Kitasato University for

32 processing.   R.W.S. then collected bone and teeth samples on-site using sterile sampling

33 methods (wearing coveralls, gloves, facemask) from four additional chambers (20, G1, G2, G3)

1   during field seasons 2015–2016. These samples were directly deposited into sterile tubes and

2   are now stored at University College, Dublin. A total of 63 individual human remains were

3   analyzed for this study.

4

5   **Ancient DNA extraction**

6

7   DNA extraction was carried out with ~100–300 mg of bone powder using a modified

8   silica-column based protocol (Yang et al., 1998; Gamba et al., 2014; 2015). First, the surface

9   of the bone was cleaned using a diamond drill bit a low speed. Then, we either used the drill

10   bit to obtain powder, or powdered the bone in a mixer mill, ShakeMaster Auto ver.2.0

11   (BioMedical Science Inc.). For samples VC001-VC035 and VC046-VC063, we used the

12   following protocol: bone powder was incubated for 24 h at 55°C followed by 24 h at 37°C in 2

13   ml tubes with 1 ml of lysis buffer in final concentrations of Tris HCL pH 7.4 20mM; Sarkosyl

14   NL 0.7%; 0.5 M EDTA pH 8.0 47.5mM; Proteinase K 0.65U/ml, shaking at 300 rpm in a

15   Thermomixer (Thermomixer comfort Eppendorf®). Samples were then centrifuged at 13,000

16   rpm for 10 m and the supernatant was removed. Fresh lysis buffer (1 ml) was then added to

17   the pellet, vortexed, and the incubation and centrifugation steps were repeated.

18   The second supernatant was then transferred to an Amicon® Ultra-4 Centrifugal Filter Unit

19   30K, diluted with 3 ml of TE and centrifuged at ~2,500 rpm until a final concentration of ~100

20   μl was obtained. This volume was then transferred to a silica column (MinElute PCR

21   Purification Kit, QIAGEN) and purified according to manufacturers instructions, except at the

22   final step adding TWEEN 20 (at 0.05% final concentration) to 60 μl EB buffer pre-heated to

23   60℃.

24   For samples VC036-VC044, our second protocol followed the first with the following

25   modifications based on a "pre-digestion" step recommended in Gamba et al. (2015): Fresh lysis

26   buffer was added to the powder and incubated in a Thermomixer for 1 h at 56°C shaking at

27   1200 rpm. After 1 h, the sample was centrifuged for 2 m at 13,000 rpm and the supernatant

28   was discarded. Fresh lysis buffer was then added to the pellet and incubated at 56°C for 1 h

29   followed by 37°C overnight, shaking at 1200 rpm. After ultra-filtration in an Amicon®

30   Ultra-4 Centrifugal Filter Unit 30K, the sample was transferred to a silica column (MinElute

31   PCR Purification Kit, QIAGEN) with the following modifications: After adding the PB buffer,

32   the centrifugation speed was reduced to 8,000 rpm and the elution step included a 5 m

33   incubation at room temperature.

1

Contamination Controls

3

4   To control for contamination, all pre-PCR procedures were conducted in a controlled-access,

5   positive pressure laboratory with HEPA-filtered air that is installed exclusively for ancient DNA

6   analysis at Kitasato University.   Disposable protective clothing was worn during all sampling

7   and extraction procedures.   Pipettes with aerosol-resistant tips were used. The lab is cleaned

8   prior to all procedures using DNA-OFF™ (Takara, Japan), and exposed to UV irradiation for at

9   least two hours after each procedure.   All PCR reactions and post-PCR procedures were

10   performed in a separate laboratory.   The movement of laboratory materials and personnel was

11   always unidirectional, from the ancient to modern facilities.   The mtDNA of all researchers

12   with access to the clean lab were typed and compared with the results.   At least two extractions

13   and two amplifications were performed at separate times to assess the authenticity of our

14   results.

15

PCR amplification and direct sequencing

17

18   The hypervariable region I (HVRI) of the mitochondrial D-loop (367 base pairs, nucleotide

19   positions 15999 – 16366) was targeted for amplification using three sets of overlapping primers

20   (Table 1).   PCR amplification was carried out using 2 µl extract in a 50 µl reaction mixture

21   containing Ex Taq Hot Start, 10x Ex Taq buffer (containing $MgCl_2$), 2.5 mM dNTPs, and 10uM

22   each primer.   PCR conditions were 94°C for 5 min followed by 40 cycles of 94°C for 30 sec,

23   55°C and 61.5°C for Primer Set 1and Primer Sets 2/3, respectively, annealing temperature for

24   30 sec, 72°C for 30 sec, extension of 72°C for 5 min, and a hold at 4°C.   A negative control

25   was included in each PCR run.   PCR products were visualized on 2% agarose gel containing

26   ethidium bromide, and purified using the MinElute PCR Purification Kit, QIAGEN.

27   Amplification products were sequenced using Applied Biosystems Big Dye protocols and

28   analyzed on an Applied Biosystems 3130 Genetic Analyzer at Kitasato University.

29

Data analysis

31

32   Sequences were visually inspected, corrected and compared against the revised Cambridge

33   Reference Sequence (rCRS) (Andrews et al. 1999) using DNASTAR® Lasergene software

14

1   (SeqMan Pro).   Maximum-parsimonious phylogenetic network was constructed by hand.

2   HVS-I haplotypes were classified into possible haplogroups and sub-haplogroups using

3   MITOMAP (Lott et al. 2013).   We compared the frequencies of haplogroups found in VC with

4   ancient and modern people (58 populations / 14,735 individuals).   Using the nucleotide

5   sequence information obtained this time and the known nucleotide sequence information

6   analyzed in [39] (29 populations / 1846 individuals), population genetic statistics, the number of

7   sequence types, the number of polymorphic sites, the nucleotide diversity (π), Tajima's D, were

8   calculated by software, DnaSP 5.

9

10

11   **References**

12

13   [1]   Haak W, Balanovsky O, Sanchez JJ, Koshel S, Zaporozhchenko V, Adler CJ, et al.

14   Ancient DNA from European Early Neolithic Farmers Reveals Their Near Eastern

15   Affinities. PLoS Biol. 2010;8:e1000536.

16

17   [2]   Bollongino R, Nehlich O, Richards MP, Orschiedt J, Thomas MG, Sell C, et al. 200

18   years of parallel societies in Stone Age Central Europe. Science. 2013;342:479-481.

19

20   [3]   Allentoft ME, Sikora M, Sjögren KG, Rasmussen S, Rasmussen M, Stenderup J, et al.

21   Population genomics of Bronze Age Eurasia. Nature. 2015;522:167-172.

22

23   [4]   Mathieson I, Lazaridis I, Rohland N, Mallick S, Patterson N, Roodenberg SA, et al.

24   Genome-wide   patterns   of   selection   in   230   ancient   Eurasians.   Nature.

25   2015;528:499-503.

26

27   [5]   Brandt G, Szécsényi-Nagy A, Roth C, Alt KW, Haak W. Human paleogenetics of

28   Europe – The known knowns and the known unknowns. J Hum Evol. 2015;79:73-92.

29

30   [6]   Jones ER, Gonzalez-Fortes G, Connell S, Siska V, Eriksson A, Martiniano R, et al.

31   Upper Palaeolithic genomes reveal deep roots of modern Eurasians. Nat Commun.

32   2015;6:8912.

33

1   [7]   Ammerman AJ, Cavalli-Sforza LL. The Neolithic transition and the genetics of
2   populations in Europe. Princeton: Princeton University Press; 1984.
3
4   [8]   Pinhasi R, von Cramon-Taubadel N. Craniometric data supports demic diffusion model
5   for the spread of agriculture into Europe. PLoS ONE. 2009;4:e6747. doi:10.
6   1371/journal.pone.0006747.
7
8   [9]   Cavalli-Sforza LL. Genes, peoples, and languages. Berkeley and Los Angeles:
9   University of California Press; 2000.
10
11 [10] Haber M, Mezzavilla M, Xue Y, Tyler-Smith C. Ancient DNA and the rewriting of
12   human history: be sparing with Occam's razor. Genome Biol. 2016;17:1. doi:
13   10.1186/s13059-015-0866-z.
14
15 [11] Sampietro M, Lao O, Caramelli D, Lari M, Pou R, Marti M, et al. Palaeogenetic
16   evidence supports a dual model of Neolithic spreading into Europe. Proc Biol Sci.
17   2007;274:2161-2167.
18
19 [12] Lacan M, Keyser C, Ricaut FX, Brucato N, Duranthon F, Guilaine J, et al. Ancient
20   DNA reveals male diffusion through the Neolithic Mediterranean route. Proc Nat Acad
21   Sci USA. 2011a;108:9788-9791.
22
23 [13] Lacan M, Keyser C, Ricaut FX, Brucato N, Tarrus J, Bosch A, et al. Ancient DNA
24   suggests the leading role played by men in the Neolithic dissemination. Proc Nat Acad
25   Sci USA. 2011b;108:18255-18259.
26
27 [14] Hervella M, Izagirre N, Alonso S, Fregel R, Alonso A, Cabrera VM, et al. Ancient
28   DNA from hunter-gatherer and farmer groups from Northern Spain supports a random
29   dispersion model for the Neolithic expansion into Europe. PLoS ONE. 2012;7:e34417.
30
31 [15] Zvelebil M, Dolukhanov P. The transition to farming in Eastern and Northern Europe. J
32   World Prehist. 1991;5:233-278.
33

[16] Ryzhov SN. Relative chronology of the giant-settlement period BII-CI. In: Menotti F, Korvin-Piotrovskiy AG, editors. Tripolye Culture: giant settlements in Ukraine: formation, development, and decline. Oxford: Oxbow Books; 2012. p. 79-115.

[17] Anthony DW. The horse, the wheel, and language: how Bronze Age riders from the Eurasian steppes shaped the modern world. Princeton: Princeton University Press; 2007.

[18] Karsten JK, Heins SE, Madden GD, Sokhatsky MP. The Biological Implications of the Transition to Agriculture in Ukraine: A Study of Enamel Hypoplasias. Dental Anthropology. 2014;27:16-25.

[19] Karsten JK, Heins SE, Madden GD, Sokhatsky MP. Dental health and the transition to agriculture in prehistoric Ukraine: A study of dental caries. Eur J Archaeol. 2015;18:562-579.

[20] Nikitin AG, Sokhatsky MP, Kovaliukh M, Videiko MY. Comprehensive site chronology and ancient mitochondrial DNA analysis from Verteba cave – a Trypillian culture site of Eneolithic Ukraine. Interdiscip Archaeol. 2010;1:9-18.

[21] Nikitin AG, Potekhina I, Rohland N, Mallick S, Reich D, Lillie, M. Mitochondrial DNA analysis of eneolithic trypillians from Ukraine reveals neolithic farming genetic roots. PLoS ONE. 2013;12(2):e0172952. doi:10.1371/journal. pone.0172952.

[22] Nikitin AG. Bioarchaeological analysis of Bronze Age human remains from the Podillya region of Ukraine. Interdiscip Archaeol. 2011;2:9-14.

[23] Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet. 1999;23:147.

[24] Tajima F. Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. Genetics. 1989;123:585-595.

17

[25]   Aris-Brosou S, Excoffier L. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol Biol Evol. 1996;13(3):494-504.

[26]   Richards M, Macaulay V, Torroni A, Bandelt HJ. In search of geographical patterns in European mitochondrial DNA. Am J Hum Genet. 2002;71:1168-1174.

[27]   Haak W, Lazaridis I, Patterson N, Rohland N, Mallick S, Llamas B, et al. Massive migration from the steppe was a source for Indo-European languages in Europe. Nature. 2015;522:207-211.

[28]   Jones ER, Zarina G, Moiseyev V, Lightfoot E, Nigst PR, Manica A, et al. The Neolithic Transition in the Baltic Was Not Driven by Admixture with Early European Farmers. Curr Biol. 2017;27(4):576-582.

[29]   Van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009;30:E386-E394.

[30]   Malyarchuk BA, Derenko MV. Mitochondrial DNA variability in Russians and Ukrainians: Implication to the origin of the Eastern Slavs. Ann Hum Genet. 2001;65:63-78.

[31]   Nikitin AG, Kochkin IT, June CM, Willis CM, Mcbain I, Videiko MY. Mitochondrial DNA sequence variation in the Boyko, Hutsul, and Lemko populations of the Carpathian highlands. Hum Biol. 2009;81:43-58.

[32]   Deguilloux M, Mendisco F. Ancient DNA: A window to the past of Europe. Hum Hered. 2013;76:121-132.

[33]   Posth C, Renaud G, Mittnik A, Drucker DG, Rougier H, Cupillard C, et al. Pleistocene Mitochondrial Genomes Suggest a Single Major Dispersal of Non-Africans and a Late Glacial Population Turnover in Europe. Curr Biol. 2016;26:827-833.

[34] Muller J, Rassmann K, Videiko M. Trypillia Mega-Sites and European Prehistory: 4100–3400 BCE. New York: Routledge; 2016.

[35] Wilde S, Timpson A, Kirsanow K, Kaiser E, Kayser M, Unterlander M, et al. Direct evidence for positive selection of skin, hair, and eye pigmentation in Europeans during the last 5,000 y. Proc Natl Acad Sci USA. 2014;111:4832-4837.

[36] Kristiansen K, Allentoft ME, Frei KM, Iversen R, Johannsen NN, Kroonen G, et al. Re-theorising mobility and the formation of culture and language among the Corded Ware Culture in Europe. Antiquity. 2017;91(356):334-347.

[37] Watson E, Bauer K, Aman R, Weiss G, von Haeseler A, Paabo S. mtDNA sequence diversity in Africa. Am J Hum Genet. 1996;59:437-444.

[38] Oota H, Kitano T, Jin F, Yuasa I, Wang L, Ueda S, et al. Extreme mtDNA Homogeneity in Continental Asian Populations. Am J Phys Anthropol. 2002;118:146-153.

[39] Ledogar SH, Karsten JK, Madden GD, Sokhatsky M, Schmidt RW, Feranec RS. New Chronology and Reinterpretation of Eneolithic deposits from Verteba Cave (Ternopil Region, Ukraine). Radiocarbon. In press.

[40] Madden GD, Karsten JK, Heins SE, Sokhatsky M, Schmidt R. Violence at Verteba Cave: Cranial Trauma as Evidence for Intergroup Conflict in Late Neolithic Ukraine. Int J Osteoarchaeol. In press.

[41] Rasmussen S, Allentoft ME, Nielsen K, Orlando L, Sikora M, Sjogren K, et al. Early Divergent Strains of Yersinia pestis in Eurasia 5,000 Years Ago. Cell. 2015;163:571-582.

[42] Mathieson I, Roodenberg SA, Posth C, Szecsenyi-Nagy A, Rohland N, Mallick S, et al. The genomic history of Southeastern Europe. BioRxiv, posted 19 September 2017.

[43] Achilli A, Olivieri A, Pala M, Metspalu E, Fornarino S, Battaglia V, et al. Mitochondrial DNA Variation of Modern Tuscans Supports the Near Eastern Origin of Etruscans. Am J Hum Genet. 2007;80:759–768.

[44] Alt KW, Knipper C, Peters D, Müller W, Maurer A, Kollig I, et al. Lombards on the Move - An integrative study of the migration period cemetery at Szólád, Hungary. PLoS One. 2002;doi: 10.1371/journal.pone.0110793.

[45] Badro DA, Douaihy B, Haber M, Youhanna SC, Salloum A, Ghassibe-Sabbagh M, et al. Y-Chromosome and mtDNA Genetics Reveal Significant Contrasts in Affinities of Modern Middle Eastern Populations with European and African Populations. PLoS One. 2013;doi: 10.1371/journal.pone.0054616

[46] Bamshad M, Kivisild T, Watkins WS, Dixon ME, Riker CE, Rao BB, et al. Genetic evidence on the origins of Indian caste populations. Genome Res. 2001;11:994–1004.

[47] Bermisheva MA, Tambets K, Villeins R, Khusnutdinova EK. Diversity of mitochondrial DNA haplogroups in ethnic populations of the Volga-Ural region. Mol Biol. 2004;36:802–812.

[48] Bogácsi-Szabó E, Csányi B, Tömöry G, et al. Archeogenetikai vizsgálatok a Kárpát-medence X. századi népességén. Magy Tudomány. 2008;10:1204–1217.

[49] Bogácsi-Szabó E, Kalmár T, Csányi B, et al. Mitochondrial DNA of ancient Cumanians: culturally Asian steppe nomadic immigrants with substantially more western Eurasian mitochondrial DNA lineages. Hum Biol an Int Rec Res. 2005;77:639–662.

[50] Bosch E, Calafell F, González-Neira A, Flaiz C, Meteu E, Scheil HG, et al. Paternal and maternal lineages in the Balkans show a homogeneous landscape over linguistic barriers, except for the isolated Aromuns. Ann Hum Genet. 2006;70:459–487.

[51] Brandstatter A, Egyed B, Zimmermann B, Duftner N, Padar Z, Parson W. Migration

20

rates and genetic structure of two Hungarian ethnic groups in Transylvania, Romania. Ann Hum Genet. 2007;71:791–803.

[52] Csákyová V, Szécsényi-Nagy A, Csosz A, Nagy M, Fusek G, Langó et al. Maternal genetic composition of a medieval population from a Hungarian-Slavic contact zone in central Europe. PLoS One. 2016;doi: 10.1371/journal.pone.0151206.

[53] Der Sarkissian C. Mitochondrial DNA in ancient human populations of Europe. University of Adelaide. 2011.

[54] Derbeneva OA, Starikovskaia EB, Volod'ko N V, et al. Mitochondrial DNA variation in Kets and Nganasans and the early peoples of Northern Eurasia. Genetika. 2002;38:1554–1560.

[55] Derenko M, Malyarchuk B, Bahmanimehr A, Denisova G, Perkova M, Farjadian S, Yepiskoposyan L. Complete mitochondrial DNA diversity in Iranians. PLoS One. 2013;doi: 10.1371/journal.pone.0080673.

[56] Derenko M, Malyarchuk B, Grzybowski T, Denisova G, Dambueva I, Perkova M, et al. Phylogeographic analysis of mitochondrial DNA in northern Asian populations. Am J Hum Genet. 2007;81:1025–41.

[57] Di Cristofaro J, Pennarun E, Mazières S, Myres NM, Lin AA, Temori SA, et al. Afghan Hindu Kush: Where Eurasian Sub-Continent Gene Flows Converge. PLoS One. 2013;doi: 10.1371/journal.pone.0076748.

[58] Egyed B, Brandstatter A, Irwin JA, Pádár Z, Parsons TJ, Parson W. Mitochondrial control region sequence variations in the Hungarian population: Analysis of population samples from Hungary and from Transylvania (Romania). Forensic Sci Int Genet. 2007;1:158–162.

[59] Gonzalez AM, Brehm A, Pérez JA, Maca-Meyer N, Flores C, Cabrera VM. Mitochondrial DNA affinities at the Atlantic fringe of Europe. Am J Phys Anthropol.

21

1   2003;120:391–404.

2

3   [60]   González-Ruiz M, Santos C, Jordana X, Simón M, Lalueza-Fox C, Gigli E, et al.
4   Tracing the Origin of the East-West Population Admixture in the Altai Region (Central
5   Asia). PLoS One. 2012;doi: 10.1371/journal.pone.0048904.

6

7   [61]   Gresham D, Morar B, Underhill PA, Passarino G, Lin AA, Wiser C, et al. Origins and
8   divergence of the Roma (gypsies). Am J Hum Genet. 2001;69:1314–31.

9

10   [62]   Guimaraes S, Ghirotto S, Benazzo A, Milani L, Lari M, Pilli E, et al. Genealogical
11   discontinuities among Etruscan, medieval, and contemporary Tuscans. Mol Biol Evol.
12   2009;26:2157–2166.

13

14   [63]   Helgason A, Hickey E, Goodacre S, Bosnes V, Stefánsson K, Ward R, Sykes B.
15   mtDNA and the islands of the North Atlantic: estimating the proportions of Norse and
16   Gaelic ancestry. Am J Hum Genet. 2001;68:723–737.

17

18   [64]   Hollard C, Keyser C, Giscard PH, Tsagaan T, Bayarkhuu N, Bemmann J, Crubézy E,
19   Ludes B. Strong genetic admixture in the Altai at the Middle Bronze Age revealed by
20   uniparental and ancestry informative markers. Forensic Sci Int Genet. 2014;12:199–
21   207.

22

23   [65]   Ingman M, Gyllensten U. A recent genetic link between Sami and the Volga-Ural
24   region of Russia. Eur J Hum Genet. 2007;15:115–20.

25

26   [66]   Irwin JA, A. Ikramov, J. Saunier, M. Bodner, S. Amory, A. Röck. The mtDNA
27   composition of Uzbekistan: a microcosm of Central Asian patterns. Int. J. Legal Med.
28   2010;124:195–204.

29

30   [67]   Keyser-Tracqui, Crubézy E, Ludes B. Nuclear and Mitochondrial DNA Analysis of a
31   2,000-Year-Old Necropolis in the Egyin Gol Valley of Mongolia Am J Hum Genet.
32   2003;73:247-60.

33

1   [68]   Keyser C, Bouakaze C, Crubuzy E, Nikolaev VG, Montagnon D, Reis T, Ludes.
2   Ancient DNA provides new insights into the history of south Siberian Kurgan people.
3   Hum Genet. 2009;126:395–410.
4
5   [69]   Krzewinska M, Bjornstad G, Skoglund P, Olason PI, Bill J, Götherström A, Hagelberg
6   E. Mitochondrial DNA variation in the Viking age population of Norway. Philos Trans
7   R Soc London Ser B Biol Sci. 2015;370:20130384.
8
9   [70]   Kushniarevich A, Sivitskaya L, Danilenko N, Novogrodskii T, Tsybovsky I, Kiseleva A,
10   et al. Uniparental Genetic Heritage of Belarusians: Encounter of Rare Middle Eastern
11   Matrilineages with a Central European Mitochondrial DNA Pool. PLoS One. 2013;doi:
12   10.1371/journal.pone.0066499.
13
14 [71]   Lalueza-Fox C, Sampietro ML, Gilbert MTP, Castri L, Facchini F, Pettener D,
15   Bertranpetit J. Unravelling migrations in the steppe: mitochondrial DNA sequences
16   from ancient central Asians. Proc Biol Sci, 2004;271:941–7.
17
18 [72]   Lazaridis I Nadel D, Rollefson G, Merrett DC, Rohland N, Mallick S, et al. Genomic
19   insights into the origin of farming in the ancient Near East. Nature. 2016; 536:419-424.
20
21 [73]   Li C, Ning C, Hagelberg E, Li H, Zhao Y, Li W, Abudurseule I, Zhu H, Zhou H.
22   Analysis of ancient human mitochondrial DNA from the Xiaohe cemetery: insights into
23   prehistoric population movements in the Tarim Basin, China. BMC Genet. 2015;16:78.
24   doi: 10.1186/s12863-015-0237-5.
25
26 [74]   Malyarchuk BA, Grzybowski T, Derenko MV, Czarny J, Drobnič, Miścicka-Śliwka.
27   Mitochondrial DNA variability in Bosnians and Slovenians. Ann Hum Genet.
28   2003;67:412–425.
29
30 [75]   Malyarchuk BA, Perkova MA, Derenko MV. On the origin of Mongoloid component in
31   the mitochondrial gene pool of Slavs. Russ J Genet. 2008;44:344–349.
32
33 [76]   Maruyama S, Minaguchi K, Saitou N. Sequence polymorphisms of the mitochondrial

DNA control region and phylogenetic analysis of mtDNA lineages in the Japanese population. Int J Legal Med. 2003;117:218–225.

[77]   Melchior L, Kivisild T, Lynnerup N, Dissing J. Evidence of authentic DNA from Danish Viking Age skeletons untouched by humans for 1,000 years. PLoS One. 2008;doi: 10.1371/journal.pone.0002214.

[78]   Molodin VI, Pilipenko AS, Romaschenko AG, et al. Human migrations in the southern region of the West Siberian Plain during the Bronze Age: Archaeological, palaeogenetic and anthropological data. In: Population Dynamics in Prehistory and Early History: New Approaches Using Stable Isotopes and Genetics. 2012; pp 93–112.

[79]   Morozova I, Evsyukov A, Kon'Kov A, Grosheva A, Zhukova O, Rychkov S. Russian ethnic history inferred from mitochondrial DNA diversity. Am J Phys Anthropol. 2012;147:341–351.

[80]   Newton J. Ancient Mitochondrial DNA From Pre-historic Southeastern Europe: The Presence of East Eurasian Haplogroups Provides Evidence of Interactions with South Siberians Across the Central Asian Steppe Belt. Grand Valley State University. 2011.

[81]   Passarino G, Semino O, Quintana-Murci L, Excoffier L, Hammer M, Santachiara-Benerecett A. Different genetic components in the Ethiopian population, identified by mtDNA and Y-chromosome polymorphisms. Am J Hum Genet. 1998;62:420–34.

[82]   Pereira L, Prata MJ, Amorim. Diversity of mtDNA lineages in Portugal: not a genetic edge of European variation. Ann Hum Genet. 2000;64:491–506.

[83]   Pericić M, Barać Lauc L, Martinović Klarić I, Janićijević B, Rudan P. Review of Croatian genetic heritage as revealed by mitochondrial DNA and Y chromosomal lineages. Croat Med J. 2005;46(4):502-13.

[84]   Pilipenko AS, Romaschenko AG, Molodin VI, Parsinger H, Kobzev VF. Mitochondrial

24

DNA studies of the Pazyryk people (4th to 3rd centuries BC) from northwestern Mongolia. Archaeol Anthropol Sci. 2010;2:231–236.

[85]   Pimenoff VN, Comas D, Palo JU, Vershubsky G, Kozlov A, Sajantila A. Northwest Siberian Khanty and Mansi in the junction of West and East Eurasian gene pools as revealed by uniparental markers. Eur J Hum Genet. 2008;16:1254–64.

[86]   Pliss L, Tambets K, Loogvoli EL, Pronina N, Lazdins M, Krumina A, Baumanis V, Villems R. Mitochondrial DNA portrait of Latvians: Towards the understanding of the genetic structure of Baltic-speaking populations. Ann Hum Genet. 2006;70:439–458.

[87]   Quintana-Murci L, Chaix R, Wells RS, Behar DM, Sayar H, Scozzari R, et al. Where west meets east: the complex mtDNA landscape of the southwest and Central Asian corridor. Am J Hum Genet. 2004;74:827–45.

[88]   Ricaut FX, Keyser-Tracqui C, Bourgeois J, Crubézy E, Ludes B. Genetic analysis of a Scytho-siberian skeleton and its implications for ancient Central Asian migrations. Hum Biol. 2004;76:109–125.

[89]   Starikovskaya EB, Sukernik RI, Derbeneva OA, Volodko NV, Ruiz-Pesini E, Torroni A, et al. Mitochondrial DNA diversity in indigenous populations of the southern extent of Siberia, and the origins of Native American haplogroups. Ann Hum Genet. 2005;69:67–89.

[90]   Szécsényi-Nagy A, Brandt G, Haak W, Keerl V, Jakucs J, Möller-Rieker S, et al. Tracing the genetic origin of Europe's first farmers reveals insights into their social organization. Philos Trans R Soc B Biol Sci. 2015;282:20150339.

[91]   Tömöry G, Csányi B, Bogácsi-Szabó E, Kalmár T, Czibula A, Csösz A et al. Comparison of maternal lineage and biogeographic analyses of ancient and modern Hungarian populations. Am J Phys Anthropol. 2007;134:354–368.

[92]   Trapezov RO, Pilipenko AS, Molodin VI. Mitochondrial DNA diversity in the gene

pool of the Neolithic and Early Bronze Age Cisbaikalian human population. Russ J Genet Appl Res. 2015;5:26–32.

[93]   Vai S, Ghirotto S, Pilli E, Tassi F, Lari M,, Rizzi E, et al. Genealogical Relationships between Early Medieval and Modern Inhabitants of Piedmont. PLoS One. 2015;10:e0116801. doi: 10.1371/journal.pone.0116801

[94]   Volodko N V., Starikovskaya EB, Mazunin IO, Eltsov NP, Naidenko PV, Wallace DC, Sukernick RI. Mitochondrial Genome Diversity in Arctic Siberians, with Particular Reference to the Evolutionary?? History of Beringia and Pleistocenic Peopling of the Americas. Am J Hum Genet. 2008;82:1084–1100.

[95]   Wen B, Xie X, Gao S, Li H, Shi H, Song X, et al. Analyses of genetic structure of Tibeto-Burman populations reveals sex-biased admixture in southern Tibeto-Burmans. Am J Hum Genet. 2004;74:856–65.

[96]   Xu Z, Zhang F, Xu B, Tan J, Li S, Li C, et al. Mitochondrial DNA Evidence for a Diversified Origin of Workers Building Mausoleum for First Emperor of China. PLoS One. 2008;3(10): e3275. doi:10.1371/journal.pone.0003275.

[97]   Yao Y-G, Kong Q-P, Bandelt H-J, Kivisild T, Zhang YP. Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am J Hum Genet. 2002;70:635–651.

[98]   Yao YG, Kong QP, Wang CY, Zhu CL, Zhang YP. Different matrilineal contributions to genetic structure of ethnic groups in the Silk Road region in China. Mol Biol Evol. 2004;21:2265–2280.

[99]   Yosifova A, Mushiroda T, Kubo M, Takahashi A, Kamatani Y, Stoianov D, et al. Genome-wide association study on bipolar disorder in the Bulgarian population. Genes, Brain Behav. 2011;10:789–797.

[100]   Brandt G, Haak W, Alder CJ, Roth C, Szécsényi-Nagy A, Karimnia S, et al. Ancient

DNA reveals key stages in the formation of central European mitochondrial genetic diversity. Science. 2013;342:257-261.

[101] Haak W, Forster P, Bramanti B, Matsumura S, Brandt G, et al. Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. Science. 2005;310:1016-1018.

[102] Bramanti B1, Thomas MG, Haak W, Unterlaender M, Jores P, Tambets K, et al. Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. Science. 2009;326:137-140.

[103] Fu, Q Mittnik A, Johnson P, Bos K, Lari M, Bollongino R, et al. 2013. A revised timescale for human evolution based on ancient mitochondrial genomes. Curr Biol. 2013;23:553-559.

[104] Malmström, H, Gilbert MT, Thomas MG, Brandström M, Storå J, Molnar P et al. Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary Scandinavians. Curr Biol. 2009;19:1758-1762.

[105] Malmström H, Linderholm A, Skoglund P, Storå J, Sjödin P, Gilbert MT. Ancient mitochondrial DNA from the northern fringe of the Neolithic farming expansion in Europe sheds light on the dispersion process. Philos Trans R Soc Lond B Biol Sci. 2015;370:20130373.

[106] Skoglund, P Malmström H, Raghavan M, Storå J, Hall P, Willerslev E, et al. Origins and Genetic Legacy of Neolithic Farmers and Hunter-Gatherers in Europe. Science. 2012;336:466-469.

[107] Skoglund P, Malmström H, Omrak A, Raghavan M, Valdiosera C, Günther T. Genomic diversity and admixture differs for Stone-Age Scandinavian foragers and farmers. Science. 2014;344:747-750.

[108] Krause J, Briggs AW, Kircher M, Maricic T, Zwyns N, Derevianko A, Pääbo S. A complete mtDNA genome of an early modern human from Kostenki, Russia. Curr Biol.

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2010;20:231-236.

[109]   Der Sarkissian, C, Balanovsky O, Brandt G, Khartanovich V, Buzhilova A, Koshel S, et al. Ancient DNA Reveals Prehistoric Gene-Flow From Siberia in the Complex Human Population History of North East Europe. PLoS Genet. 2013;9:e1003296s.

[110]   Günther T, Valdiosera C, Malmstrom H, Ureña I, Rodriguez-Varela R, Sverrisdóttir OO, et al. Ancient genomes link early farmers from Atapuerca in Spain to modern-day Basques. Proc Natl Acad Sci USA. 2015;112:11917-11922.

[111]   Gómez-Sánchez D, Olalde I, Pierini F, Matas-Lalueza L, Gigli E, Mari M, et al. Mitochondrial DNA from El Mirador cave (Atapuerca, Spain) reveals the heterogeneity of Chalcolithic populations. PLoS One. 2014;9:e105105.

[112]   Chandler H, Sykes B, J. Zilhão. Using ancient DNA to examine genetic continuity at the Mesolithic-Neolithic transition in Portugal. In: Arias P, Ontañón R, García-Moncó C (eds.), Actas dell III Congreso del Neolítico en la Península Ibérica, Santander, Monografías del Instituto internacional de Investigaciones Prehistóricas de Cantabria 1: 2005;781-786.

[113]   Gamba C, Fernández E, Tirado M, Deguilloux MF, Pemonge MH, Utrilla P, et al. Ancient DNA from an Early Neolithic Iberian population supports a pioneer colonization by first farmers. Mol Ecol. 2012;21:45-56.

[114]   Sánchez-Quinto F Schroeder H, Ramirez O, Avila-Arcos MC, Pybus M, Olalde I, et al. Genomic Affinities of Two 7,000-Year-Old Iberian Hunter-Gatherers. Curr Biol. 2012;22:1494-1499.

[115]   Haak W, Brandt G, de Jong HN, Meyer C, Ganslmeier R, Heyd V, et al. Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age. Proc Natl Acad Sci USA. 2008;105:18226-18231.

1   Table 1. mtDNA Primers used in this study.

Primer Set	Primer	Primer's Sequence	Nucleotide positions	Product Size (bp)
1	L15998	5´- CCATTAGCACCCAAAGCTA - 3´	15980 – 16161	182
	H16142	5´- ATGTACTACAGGTGGTCAAG - 3´		
2	L16120	5´ - TTACTGCCAGCCACCATGAA - 3´	16101 – 16258	158
	H16239	5´ - TGGCTTTGGAGTTGCAGTTG - 3´		
3	L16208	5´ - CCCCATGCTTACAAGCAAG - 3´	16190 – 163383	195
	H16367	5´ - CTGAGGGGGGTCATCCAT - 3´		

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1   Table2. mtDNA nucleotide sequences of the Verteba Cave specimens

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Seq Type	Camber	Sample	Bone or Tooth	nucleotide position (+16000bp)																Predicted Haplogroup
				70	93	126	192	209	223	224	256	292	294	296	304	311	324	356	362	
		rCRS		A	T	T	C	T	C	T	C	C	C	C	T	T	T	T	T	
		K.W.		·	·	·	·	C	T	·	·	·	·	·	·	·	C	·	·	M7a
		R.S.		·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	HV12b
I	Area2 (site 7)	VC050	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H(rCRS)
		VC051	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
	7	VC016	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
		VC019	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
		VC002	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
		VC003	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
	G3	VC040	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
II	Site 7	VC001	B	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	HV12b
III	Area17 (site 7)	VC052	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H36
		VC054	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
	Site 7	VC008	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
		VC013	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
		VC035	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
	Unknown	VC056	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
		VC055	B	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
IV	Site 7	VC021	T	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	H2a
		VC015	B	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	
V	G3	VC028	T	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	W
		VC034	B	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	
		VC041	T	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	
		VC046	B	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	
		VC047	B	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	
		VC048	T	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	
		VC049	T	·	·	·	·	·	T	·	·	T	·	·	·	·	·	·	·	
VI	G2	VC038	B	·	·	·	T	·	·	·	·	·	·	·	·	·	·	C	·	HV12b
VII	G2	VC025	B	·	C	·	·	·	·	C	·	·	·	·	·	·	C	·	·	K1a
	20	VC032	B	·	C	·	·	·	·	C	·	·	·	·	·	·	C	·	·	
VIII	Site 7	VC004	B	·	·	C	·	·	·	·	T	·	T	T	C	·	·	·	C	T2b

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1   Table3. Classification of geographical populations and genetic diversity statistics

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Area	Name of Population	Size	Number of Sequence Types	Number of Segregating Sites(S)	Nucleotide Diversity(π)	Tajima's D	
	Verteba Cave	**28**	**8**	**14**	**0.00634**	**-1.18985**	
		11	**8**	**14**	**0.00919**	**-1.28896**	
West Europe （N=595）	Basques	45	27	31	0.00894	-1.86317	*
	British	171	65	67	0.01189	-1.95117	*
	Danish	31	25	28	0.01957	-0.83873	
	Germany	107	73	59	0.01726	-1.89550	*
	Icelanders	39	29	32	0.01404	-1.30829	
	Italy Toskany	49	40	55	0.01343	-2.05834	*
	N_Spain	30	26	35	0.01446	-1.90059	*
	Portugal	54	37	39	0.01167	-1.97766	*
	Sardinian	69	45	52	0.01180	-2.05228	*
North Europe （N=255）	Estonia	26	23	32	0.01280	-1.69668	*
	Finns	48	33	35	0.01045	-1.83038	*
	Norway	82	17	20	0.00969	-0.78572	
	Sweden	25	11	17	0.00909	-0.98565	
	Switzerland	74	42	43	0.00971	-1.94697	*
East Europe （N=29)	Bulgaria	29	22	39	0.01226	-1.98753	*
West Asia （N=71）	Anatolia Turks	45	40	55	0.01482	-2.03925	*
	Turks	26	24	52	0.01812	-1.96694	*
Central Asia （N=205)	Kazakh	55	45	62	0.01764	-1.87704	*
	Kirghiz Sary Tash	47	34	56	0.01613	-1.95020	*
	Kirghiz Talas	48	43	55	0.01762	-1.81413	*
	Uighurs	55	45	61	0.01602	-2.04502	*
South Asia （N=66)	Indian	66	35	54	0.01614	-1.59922	
Northeast Asia （N=32）	Russia Siberia	16	14	23	0.01478	-1.10856	
	Siberia Altai	16	16	27	0.01513	-1.47523	
East Asia （N=593)	Cantonese	18	18	36	0.01745	-1.53414	
	Changsha	82	69	70	0.01821	-1.92022	*
	Mongolian	103	82	79	0.01765	-1.90508	*
	Korean	306	200	127	0.01458	-2.32106	*
	Xian	84	76	63	0.01784	-1.81700	*

3   * = p < 0.05

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1   Table 4. Modern Population Comparative Haplogroup Frequencies

Area	Region	n	W	HV*	H	T	K	SUM(VC)	others	total	Source
	Verteba Cave	28	25.0%	7.1%	57.1%	3.6%	7.1%	99.9%	0.0%	99.9%	This Study
	Verteba Cave	11	9.1%	18.2%	45.5%	9.1%	18.2%	100.0%	0.0%	100%	This Study
Wset Europe	Balkans	2806	2.5%	4.1%	41.2%	8.7%	5.6%	62.1%	36.6%	98.7%	Badro 2013, Bosch 2006, Gresham 2001, Malyarchuk 2003, Pericić 2005
	British Isles	1662	1.1%	0.0%	44.5%	9.5%	8.5%	63.7%	36.3%	100.0%	González 2003, Helgason 2001
	West Europe	1464	1.2%	2.3%	48.2%	8.7%	7.9%	68.4%	31.6%	100.0%	Badro 2013, González 2003, Helgason 2001
	Iberia	376	1.6%	0.0%	48.9%	8.2%	5.3%	64.1%	35.8%	99.8%	González 2003, Pereira 2000
	Italy	322	2.2%	7.2%	39.1%	11.2%	6.8%	66.5%	33.0%	99.5%	Achili 2007
East Europe	E. Europe (All)	2126	2.4%	2.4%	41.7%	8.7%	3.6%	58.9%	40.9%	99.7%	Kushniarevich 2013, Malyarchuk 2001, Morozova 2011, Pliss 2006
	Ukraine	18	0.0%	0.0%	44.4%	16.6%	5.5%	66.5%	33.3%	99.8%	Malyarchuk and Derenko 2001
Central Europe	C. Europe (All)	1496	3.8%	3.4%	40.6%	10.3%	5.9%	64.0%	35.9%	99.9%	Egyed 2007, Malyarchuk 2003, Malyarchuk 2008, Brandstatter 2007
North Europe	Scandinavia	1136	1.4%	0.0%	26.0%	5.6%	3.8%	36.8%	62.8%	99.6%	González 2003, Helgason 2001, Ingman 2007, Passarino 2002
Central Asia	Central Asia	350	2.0%	8.3%	14.8%	3.4%	2.0%	30.5%	68.0%	98.5%	Di Cristofaro 2013, Yao 2004
West Asia	Caucasus	168	1.8%	4.8%	10.1%	5.9%	4.7%	27.3%	72.6%	99.9%	Quintana-Murci 2005, Derenko 2007
	Middle East	2646	1.5%	5.5%	21.1%	8.9%	6.8%	43.9%	55.7%	99.5%	Badro 2013, Derenko 2007, Quintana-Murci 2004
	Greater Iran	533	2.8%	9.2%	18.2%	4.8%	3.9%	38.9%	61.0%	99.9%	Di Cristofaro 2013, Quintana-Murci 2004
East Asia	East Asia	617	0.5%	0.5%	0.8%	0.3%	0.3%	2.4%	97.6%	100.1%	Derenko 2007, Maruyama 2003, Yao 2002 , Yao 2004
Northeast Asia	Northern Siberia	828	0.1%	0.0%	0.7%	0.0%	0.2%	1.1%	99.0%	100.1%	Derenko 2007, Starikovskaya 2005, Volodko 2008

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1 Table 5. Ancient Population Comparative Haplogroup Frequencies

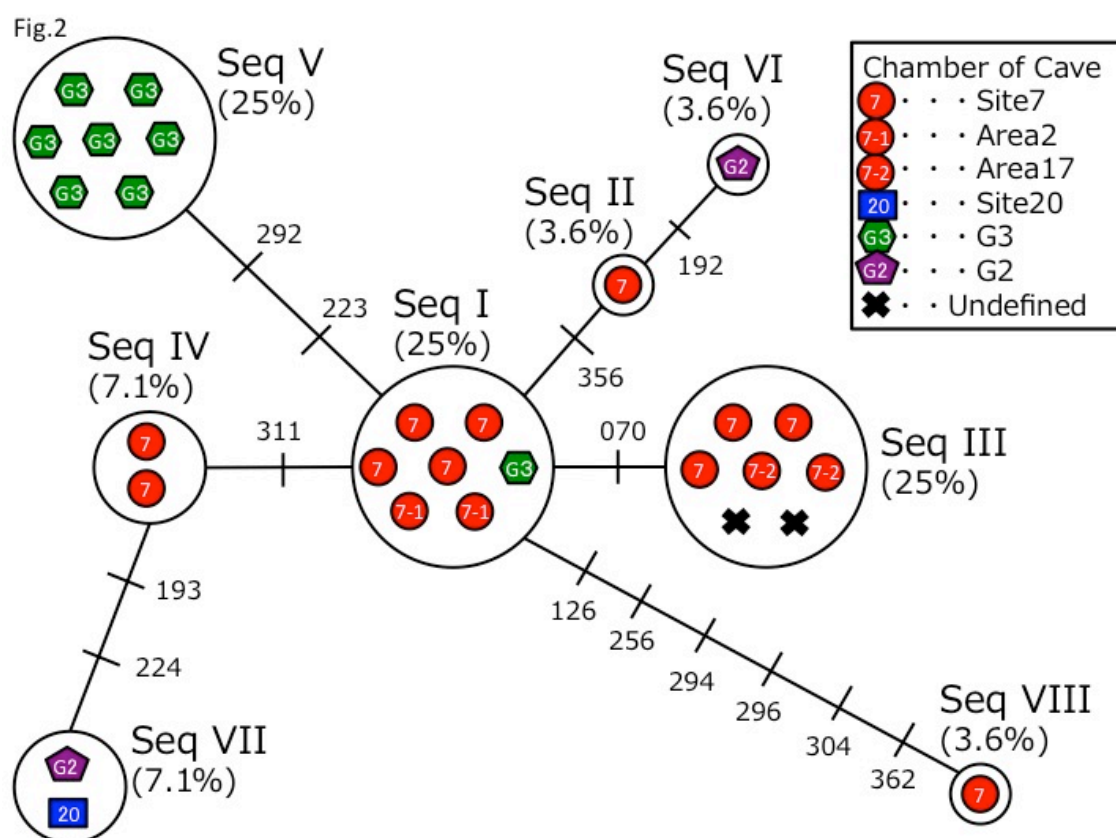| Population	n	Region	Period	W	HV	H	T	K	SUM(VC)	Others	Source
Verteba Cave	28			25.0%	7.1%	57.1%	3.6%	7.1%	99.9%	0.0%	**This Study**
Verteba Cave	11			9.1%	18.2%	45.5%	9.1%	18.2%	100.0%	0.0%	**This Study**
Starcevo	44	Balkans	Neolithic	4.6%	2.3%	6.8%	22.7%	27.3%	63.7%	36.4%	Szécsényi-Nagy 2014
Enolithic kurgans	10	BG, MD, UA	Neolithic	10.0%	0.0%	40.0%	20.0%	0.0%	70.0%	30.0%	Wilde 2014
Hungarians 900 AD	27	Central Europe	900-1000 AD	0.0%	3.7%	25.9%	14.8%	0.0%	44.4%	55.5%	Tömöry 2007
Ancient Hungarian (10th cent.)	67	Central Europe	900-1000 AD	0.0%	1.5%	32.8%	11.9%	0.0%	46.3%	53.7%	Bogácsi-Szabó 2008
Tarim Basin Xiaohe	73	China	2515-1829 BC	0.0%	0.0%	2.7%	1.4%	2.7%	6.8%	93.2%	Li 2015
Qin China	19	East Asia	221 BC-210 AD	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.1%	Xu 2008
Cisbaikalian Neol.(Serovo)	15	East Siberia	Neolithic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	Trapezov 2015
Dniepr-Donets Neolithic	17	Eastern Europe	Neolithic	0.0%	0.0%	35.3%	17.6%	0.0%	52.9%	47.1%	Newton 2011
Early-Middle Neolithic	53	Europe	Neolithic	5.7%	3.8%	26.4%	20.8%	9.4%	66.0%	34.0%	Haak 2015
Late Neolithic-EBA Europe	56	Europe	Neolithic	3.6%	1.8%	33.9%	10.7%	16.1%	66.1%	34.0%	Haak 2015; Allentoft 2015
LBK Transdanubia	39	Hungary	Neolithic	0.0%	2.6%	30.8%	28.2%	12.8%	74.4%	25.8%	Szécsényi-Nagy 2014
Szakálhát	33	Hungary	Neolithic	3.0%	3.0%	18.2%	33.4%	15.2%	72.8%	27.3%	Szécsényi-Nagy 2014
Bronze age (Vatya, Maros)	8	Hungary	Bronze Age	0.0%	0.0%	25.0%	25.0%	12.5%	62.5%	37.5%	Allentoft 2015
Ancient Hungarian Karos	17	Hungary	900-950 AD	0.0%	0.0%	17.6%	17.6%	0.0%	35.3%	64.7%	this study
Cumanian	11	Hungary	Medieval	0.0%	0.0%	36.4%	0.0%	0.0%	36.4%	63.6%	Bogácsi-Szabó 2005
Lombard early medieval	40	Hungary, Italy	500-800 AD	0.0%	2.5%	35.0%	12.5%	2.5%	52.5%	47.5%	Alt 2014, Vai 2015
Iberian Neolithic	45	Iberia	Neolithic	0.0%	2.2%	44.4%	2.2%	8.9%	57.7%	42.1%	Hervella 2012
Egyin Gol Xiongnu	46	Inner Asia	200 BC-200 AD	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	99.9%	Keyser 2003
Italian medieval	27	Italy	Medieval	0.0%	0.0%	67.0%	15.0%	3.7%	85.7%	14.8%	Guimaraes 2009
Bronze Age Kurgans	13	Kazakhstan	Bronze Age	7.7%	7.7%	30.8%	23.1%	0.0%	69.2%	30.8%	Lalueza-Fox 2004
Iron Age Kurgans	13	Kazakhstan	Iron Age	0.0%	7.7%	23.1%	7.7%	0.0%	38.5%	61.5%	Lalueza-Fox 2004
Middle East Neolithic-BrA	28	Middle East	Neolithic/Bronze Age	0.0%	0.0%	17.9%	10.7%	21.4%	50.0%	50.0%	Lazaridis 2016
Pazyryk Scytho-Siberian	25	Mongolia, Russia	Iron Age	0.0%	8.0%	12.0%	0.0%	0.0%	20.0%	80.0%	Ricaut 2004; , Pilipenko 2010; González-Ruiz 2012
Vikings	65	Norway	780-790 AD	0.0%	6.2%	40.0%	3.1%	7.7%	56.9%	43.1%	Krzewińska 2015, Melchior 2008
Srubnaya	14	Russia	Bronze Age	0.0%	0.0%	35.7%	14.3%	7.1%	57.1%	42.9%	Mathieson 2015
Tagar-Tachtyk	15	Russia	Iron Age	0.0%	6.7%	13.3%	26.7%	0.0%	46.7%	53.3%	Keyser 2009
Scythian Iron age	14	Russia	Iron Age	0.0%	0.0%	14.3%	14.3%	0.0%	28.6%	71.4%	Der Sarkissian 2011
Sintashta-Andronovo	41	Russia, Siberia	Bronze Age	0.0%	0.0%	10.0%	15.0%	5.0%	30.0%	70.0%	Keyser 2009; Allentoft 2015
Yamnaya, Afanasievo	49	Russia, Ukraine	Bronze Age	6.1%	0.0%	20.4%	20.4%	4.1%	51.0%	49.0%	Haak 2015; Allentoft 2015; Wilde 2014
Medieval Slavic	19	Slovakia	Medieval	0.0%	0.0%	21.1%	31.6%	0.0%	52.6%	47.2%	Csákyová 2016
Altai Bronze Age	12	South Siberia	Bronze Age	0.0%	0.0%	25.0%	8.0%	0.0%	33.0%	65.9%	Hollard 2014
Catacomb Kurgans	25	Ukraine	Bronze Age	0.0%	0.0%	28.0%	0.0%	0.0%	28.0%	72.0%	Wilde 2014
Baraba (UT-ODI-EK)	33	West Siberia	Bronze Age	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	Molodin 2012
Baraba (LK-FYOD-LBB)	45	West Siberia	Bronze Age	0.0%	0.0%	0.0%	15.6%	0.0%	15.6%	84.4%	Molodin 2012
Baraba (Iron transition)	14	West Siberia	Bronze Age/Iron Age	7.1%	0.0%	14.3%	0.0%	21.4%	42.9%	57.1%	Molodin 2012
Portalón	8	Spain	Chalcolithic /Bronze Age	0.0%	0.0%	25.0%	0.0%	12.5%	37.5%	62.5%	Gunther et al. 2015
Mirador	19	Spain	Chalcolithic	0.0%	0.0%	26.3%	21.1%	21.1%	68.4%	31.6%	Gomez-Sanchez et al. 2014
Neolithic Basque Country & Navarre	43	Spain	Neolithic	0.0%	2.3%	44.2%	2.3%	9.3%	58.1%	41.9%	Hervella et al. 2012
Treilles culture	29	France	Neolithic	0.0%	6.9%	20.7%	6.9%	6.9%	41.4%	58.6%	Lacan et al. 2011a
Neolithic Portugal	17	Portugal	Neolithic	0.0%	0.0%	70.6%	0.0%	0.0%	70.6%	29.4%	Chandler et al. 2005
Middle Neolithic Cardial	11	Spain	Neolithic	9.1%	0.0%	36.4%	18.2%	0.0%	63.6%	36.4%	Sampietro et al. 2007
(Epi)Cardial	18	Spain	Neolithic	0.0%	0.0%	27.8%	11.1%	33.3%	72.2%	27.8%	Gamba et al. 2012, Lacan et al. 2011b
Hunter-Gatherer south	13	Portugal, Spain	Mesolithic	0.0%	0.0%	38.5%	0.0%	0.0%	38.5%	61.5%	Chandler et al. 2005, Hervella et al. 2012, Sánchez-Quinto et al. 2012
Unetice culture	94	Germany	Bronze Age	4.3%	2.1%	21.3%	8.5%	7.4%	43.6%	56.4%	Brandt et al. 2013
Bell Beaker culture	29	Germany	Chalcolithic /Bronze Age	6.9%	0.0%	48.3%	10.3%	3.4%	69.0%	31.0%	Brandt et al. 2013
Corded Ware culture	44	Germany	Chalcolithic	2.3%	2.3%	22.7%	18.2%	13.6%	59.1%	40.9%	Haak et al. 2008, Brandt et al. 2013
Bernburg culture	17	Germany	Neolithic	5.9%	0.0%	23.5%	11.8%	17.6%	58.8%	41.2%	Brandt et al. 2013
Salzmünde culture	29	Germany	Neolithic	0.0%	3.4%	31.0%	6.9%	10.3%	51.7%	48.3%	Brandt et al. 2014
Baalberge culture	19	Germany	Neolithic	0.0%	5.3%	26.3%	26.3%	10.5%	68.4%	31.6%	Brandt et al. 2015
Schöningen group	33	Germany	Neolithic	9.1%	3.0%	15.2%	12.1%	30.3%	69.7%	30.3%	Brandt et al. 2016
Rössen culture	11	Germany	Neolithic	0.0%	9.1%	36.4%	18.2%	9.1%	72.7%	27.3%	Brandt et al. 2017
Linear Pottery culture	102	Austria, Germany	Neolithic	2.9%	4.9%	16.7%	21.6%	19.6%	65.7%	34.3%	Haak et al. 2005, Haak et al. 2010, Brandt et al. 2013
Hunter-gatherer central	16	Czech Republic, Germany, Lithuania, Luxembourg, Poland	Mesolithic	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	Bramanti et al. 2009, Fu et al. 2013
Funnel Beaker culture	15	Germany, Sweden	Mesolithic /Neolithic	0.0%	0.0%	26.7%	20.0%	13.3%	60.0%	40.0%	Malmström et al. 2009, Malmström et al. 2015, Skoglund et al. 2012, Skoglund et al. 2014, Bramanti et al. 2009
Pitted Ware culture	30	Sweden	HG/Neolithic	0.0%	0.0%	3.3%	6.7%	13.3%	23.3%	76.7%	Malmström et al. 2009, Malmström et al. 2015, Skoglund et al. 2012, Skoglund et al. 2014
Bronze Age Kazakhstan	8	Kazakhstan	Bronze Age	0.0%	12.5%	12.5%	37.5%	0.0%	62.5%	37.5%	Lalueza-Fox et al. 2004
Bronze Age Siberia	11	Russia	Bronze Age	0.0%	0.0%	9.1%	18.2%	9.1%	36.4%	63.6%	Keyser et al. 2009
Hunter-gatherer east	14	Russia	Mesolithic	0.0%	0.0%	7.1%	0.0%	0.0%	7.1%	92.9%	Bramanti et al. 2009, Krause et al. 2010, Der Sarkissian et al. 2013

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1   Figure 1. Map showing location of Verteba Cave.

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3   Figure 2. Maximum parsimonious network analysis. Circles represent the sequence types

4   ('Seq'), and the size of the circle is proportional to the number of samples.   Numbers on the

5   branches between the circles are nucleotide position numbers (+16,000) of the human

6   mitochondrial genome sequence. Sequence types correlate to mtDNA haplogroups H, HV, W,

7   K and T.

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Fig.1

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Fig.2

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