Tracking population history, social structure and intergroup exchange in Neolithic to Bronze Age Europe using ancient human and virus genomes.

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1. Introduction

The human past

People have long sought to understand human history and how it shaped the status quo. Although the idea of recording events and their causes has been around at least since Herodotus (5th century BCE), the study of human history progressed from a "backward-looking curiosity" to a scientific discipline only in the latter half of last millennium (Fagan 2018). The last few centuries have witnessed much progress in characterising the global distribution of human cultural, linguistic and biological diversity (Cunliffe 2008; Campbell 2013; Stoneking 2016), driving an increasingly better understanding of our past.

Perhaps the oldest inquiry of the human past is archaeology, which broadly refers to the study of man-made objects recovered from the past. The field's early roots are tied to antiquarians who collected and exchanged artefacts which survived from ancient and enigmatic worlds (Fagan 2018). Some of these collections grew very large and morphed into museums (e.g. the Staatliche Antikensammlungen in Munich). The field of archaeology moved from impressive collections of artefacts to systematic excavations and surveys with Stonehenge (17th century), Pompeii (1748) and Herculaneum (1738) being among the first sites to be studied. The ensuing centuries saw the development of archaeology from an amateur hobby to a fully-fledged scientific discipline, grounded in robust methods, scientific developments, and theories to interpret the human past (Renfrew & Bahn 2014; Kristiansen 2014).

The field of historical linguistics seeks to understand the differences between modern and extinct languages with the goal of elucidating their historical relationships and developments, thereby informing on past processes (Campbell 2013). The founder of the field is traditionally thought to be Sir William Jones who, in 1786 while working in India, noticed the similarities between Sanskrit and several European languages. He thereby suggested a common origin for many languages from India to Europe, becoming the first person to describe the Indo-European problem (Mallory 1989). Comparative linguistics has since matured into a field able to infer ancient sound changes, estimate divergence times between languages, deduce aspects of culture through inferred ancient lexicon, and inform on likely geographic distributions of past language through loanwords and place names (Anthony 2010).

The study of human biological diversity, physical anthropology, has dealt with the geographic distribution of biological variation (Jurmain et al., 2005). Although many aspects of phenotypic variation are subject to environmental influences, including natural and sexual selection, some are thought to be neutral and are therefore useful in inferring biological relationships between human groups (Scott & Turner 1988; Coppa et al., 1998). In addition to studying the biological relatedness between ancient groups, physical anthropology also informs on ancient demography, health (Siek 2013), incidence of violence (Jantzen et al., 2011), life-history patterns, and aspects of behaviour (Sheridan 2017).

Going molecular

Major advances came in the 20th century when human molecular variation was discovered. Initially through the analysis of blood group and protein variability (Landsteiner 1900; Von Dungerne & Hirschfield 1910; Hirschfield & Hirschfield 1919; Fisher & Taylor 1940; Mourant 1954), and later through directly assaying and quantifying DNA differences (i.e. polymorphisms), it was discovered that much human biological variation lay beneath the overt phenotypic variation (Jobling et al., 2013).

Studying molecular variation provides several advantages in comparison to phenotypic variation. Firstly, the amount of detectable molecular variation (e.g. tens of millions of genetic variants have been recently identified (Bergström et al. 2020)) greatly outnumbers phenotypic variation, allowing finer resolution when comparing individuals. Secondly, a significant amount of this variation is selectively neutral and biologically determined (Graur 2017), implying environmental factors (e.g. lifestyle, diet) throughout the course of an individual's life play no role in determining the state of these variants. As a result, the distribution of such genetic variation is largely driven by demographic events, allowing evolutionary history to be studied. Thirdly, the evolution of neutral molecular diversity follows predictable patterns, allowing for more detailed inferences of genetic split times and demographic scenarios to be deciphered (e.g. molecular clock, coalescence) (Hartl & Clark 1997).

Perhaps two of the more influential insights to have come out of the 20th century developments of molecular anthropology are the first human evolutionary trees using molecular data (Edwards & Cavalli-Sforza 1965; Cavalli-Sforza & Edwards 1967; Cavalli-Sforza et al., 1994) and the finding of a recent common origin for human mitochondrial diversity (Cann et al., 1987). By analysing protein variation, Cavalli Sforza and colleagues were able to quantify genetic distances between populations, which they used to reconstruct a tentative migratory history of our species. Part of this work suggested that the spread of agriculture to Europe was a demic process, described as a "wave of advance" progressing at ~1km/year (Ammerman & Cavalli-Sforza 1971).

By analysing worldwide variation in human mitochondrial genome variation, Cann et al. 1987 showed that the greatest diversity occurs in Africa and that the most recent common ancestor of the mitochondrial variation analysed, dubbed "Mitochondrial Eve", likely existed in Africa around 200,000 years ago. With many debates between proponents of a recent African vs a multiregional origin of Homo Sapiens ongoing, this work provided overwhelming support for the 'recent out of Africa' hypothesis of human origins (Stringer 2011), which, despite recent amendments (i.e. "leaky replacement" due to low levels of archaic admixture), still explains the origin and spread of the majority of global human genetic diversity.

Although 20th century developments allowed for major advancements in our understanding of human diversity and prehistory, two hurdles proved to be major setbacks. First, human genetic variation was still analysed at a low level of resolution. The majority of studies utilised protein variation (Watkin 1956; Lewontin 1967; Cavalli-Sforza et al., 1994), restriction fragment length polymorphisms (Matullo et al., 1994; Fernandez-Santander et

al., 1999), or short stretches of DNA sequence variation (Richards et al., 1996; Macaulay et al., 1999), most commonly the hypervariable segments of the mitochondrial genome. Considering the size of the human genome, the amount of variation captured by these methods was low, resulting in low resolution insights which were oftentimes biased by the genetic marker of choice (e.g. mitochondrial DNA and its maternal inheritance) (Bandelt et al., 2006).

The second hurdle was that these studies were restricted to analysing modern-day genetic diversity. This limited analyses only to evolutionarily successful variation, which can result in biased insights into history, especially in regions of the world where relatively recent major demographic events shaped significant portions of present-day genetic variation.

Both of these hurdles have been largely overcome within the last thirty years. Firstly, the decoding of the human genome (Venter et al., 2001; Lander et al., 2001), followed by the invention of 'next-generation', or 'massively parallel', sequencing methods (Shendure & Ji 2008; Mardis 2008; Metzker 2010), have greatly expanded our knowledge of the type, function, and distribution of variation within the genomes of humans and related species (Wheeler et al., 2008; 1000 Genomes Project Consortium et al., 2010; Qin et al., 2010; Conrad et al., 2010; Pelak et al., 2010). This variation is also becoming increasingly cheaper to assay, with the cost of sequencing whole genomes having dropped from several billion to close to ~$1,000 over the last two decades (Drmanac et al., 2010; DeFranceso 2012).

Secondly, the retrieval and analysis of DNA preserved in long-dead organisms has become common practice (Orlando et al., 2021). This newly emerging field, ancient DNA (also known as palaeogenetics or archaeogenetics), has revealed unprecedented insights into (pre)historic events within the last 100,000 years (Rasmussen et al., 2010; Green et al., 2010; Reich et al., 2010; Rasmussen et al., 2011; Skoglund et al., 2012; Skoglund et al., 2014; Lazaridis et al., 2014; Haak et al., 2015; Allentoft et al., 2015; Mathieson et al., 2015; Lazaridis et al., 2016; Lipson et al., 2017; Mathieson et al., 2018; Olalde et al., 2018; Fages et al., 2019; Olalde and Posth 2020), and less commonly even up to 1,000,000 years in the past (Orlando et al., 2013; Meyer et al., 2016; van der Valk et al., 2021). Demographic and evolutionary events have been 'caught red-handed' and analysed as they happened in real time, overturning some previous inferences based on extant genetic diversity and providing much more detail on others. Some surprising findings made through directly analysing past genetic diversity include the discovery of a new archaic human lineage (Krause et al., 2010; Reich et al., 2010), the frequent finding of archaic admixture in early modern humans (Fu et al., 2015; Slon et al., 2018; Hajdinjak et al., 2021), the relatively late onset of lactase persistence (Burger et al., 2007; Gamba et al., 2014; Mathieson et al., 2015), the large and sudden recent demographic changes (Haak et al., 2015; Allentoft et al., 2015; Olalde et al., 2018), the limited genetic impact of the earliest domesticated horses on more recent domestic breeds (Fages et al., 2019; Orlando 2020), and human infection by *Yersinia pestis* millennia before historically accounted plague pandemics (Rasmussen et al., 2015; Andrades Valtueña et al., 2017; Spyrou et al., 2018; Rascovan et al., 2019).

History of ancient DNA research

The quest to extract authentic ancient DNA began in the early 1980s with experiments on museum specimens of the Quagga (~140 years old) and Egyptian mummies (~2,400 years old (Higuchi et al., 1984; Pääbo 1985). These earliest studies cloned DNA from ancient soft tissue remains into bacterial cloning vectors (plasmids), which were used as means for amplifying the ancient DNA before isolation and sequencing. Due to the enormous size of mammalian genomes, the low specificity of cloning random DNA fragments into bacterial clones, and the low number of clones able to be cultured, these early studies were difficult to reproduce and authenticate (Pääbo et al., 2004).

The invention of the polymerase chain reaction (PCR) proved a major breakthrough for many molecular biology labs (Mullis & Faloona 1987), including those interested in the recovery of ancient DNA (Pääbo et al., 2004; Rizzi et al., 2012). Through the use of specific primers efficiently targeting regions of interest in the human genome, PCR allowed for the repeated amplification and sequencing of the same genomic regions. This proved an important step forward as it allowed for multiple experiments (sometimes from independent labs) to validate results as well as the comparison of the same targeted genomic region between different ancient samples (Pääbo et al., 2004).

This breakthrough allowed researchers to study ancient DNA in a more targeted and systematic way. It soon became clear that the earliest attempts at analysing ancient DNA from the Egyptian mummy and Quagga were thwarted by what came to be known common pitfalls in ancient DNA research, namely post-mortem ancient DNA damage and modern DNA contamination. The results of both studies (Higuchi et al., 1984; Pääbo 1985) are today considered artefacts of these processes (Pääbo et al., 2004; Willerslev & Cooper 2005).

Through re-extraction and re-analysis of the Quagga museum specimen, Pääbo and Wilson (1988) found two mismatches between the published DNA sequence and their PCR amplified Quagga mtDNA sequence. They concluded that both mismatches were “cloning artefacts”, likely due to the post-mortem modification of surviving endogenous DNA molecules (Pääbo & Wilson 1988). Similarly problematic were Pääbo’s first attempts at cloning and sequencing Egyptian mummy DNA which yielded a 3,400 base pair fragment, today considered notoriously long for ancient DNA and likely the result of modern contamination (Pääbo 1985; Pääbo et al., 2004).

Not long after the first ancient DNA studies of the 1980s, numerous ambitious studies were published reporting ancient DNA millions of years old (Golenberg et al., 1990; Soltis et al., 1992; DeSalle et al., 1992; Cano et al., 1993; Poinar et al., 1993; Woodward et al., 1994). However, none of these results were reproducible (Sidow et al., 1991; Allard et al., 1995; Hedges & Schweitzer 1995; Henikoff 1995; Zischler et al., 1995; Austin et al., 1997) and, as a result, were considered unreliable. This cast some doubt over the young field of ancient DNA, which responded by imposing strict guidelines to researchers in the field (Cooper & Poinar 2000). The strict guidelines of wearing full body suits, gloves, hairnets, and boots when sampling and processing ancient material, in conjunction with the physical separation

of pre- and post-PCR facilities, played a major role in the contamination issues being controlled, allowing the field of ancient DNA to mature, providing reliable and important insights on the past.

Although faith in the authenticity of the results was restored, the low concentration of endogenous DNA coupled with the inherent requirement that PCR target molecules needed to be longer than the primers, confined ancient DNA studies to mostly analysing short regions of mitochondrial genomes (Cooper et al., 1992; Krings et al., 1997; Stone & Stoneking 1993; Hagelberg & Clegg 1993; Hagelberg et al., 1994; Höss et al., 1994; Krings et al., 1999; Ovchinnikov et al., 2000; Haak et al., 2005; Lalueza-Fox et al., 2005; Orlando et al., 2006). The presence of mitochondrial DNA in thousands of copies per cell increases its chances of preserving in an ancient specimen, however its small size and maternal inheritance result in limited and potentially skewed insights. The low concentration of ancient nuclear DNA, high degree of degradation, and requirement that PCR target molecules be longer than the primers meant that PCR amplification was restricted to fragment lengths of >80 base pairs in length, which makes up only a fraction of the available ancient DNA in an extract (Marciniak et al., 2015), rendering the reconstruction of whole ancient genomes impossible. Consequently, it was questioned whether the effort required to obtain authentic short mitochondrial segments was justified (Stoneking 1995).

The following major breakthrough came with second and (subsequently coined) 'next generation' sequencing technologies and proved revolutionary, allowing for orders of magnitude more data from ancient specimens to be analysed. These sequencing platforms, initially 454 pyrosequencing but later Illumina and others (e.g. PacBio), proved much more efficient than PCR-based and Sanger sequencing methods, allowing massively parallel sequencing of ancient DNA (Green et al., 2006; Green et al., 2010). Ancient DNA research benefited greatly from these advances, as the major advantage of this new technology was the ability to bulk sequence an entire extract and analyse all the molecules inside it, including the previously inaccessible reads shorter than ~80 base pairs in length (Marciniak et al., 2015). The orders of magnitude more data obtained from these sequencing platforms posed new challenges in processing and storing the data, with new bioinformatic file formats, pipelines, and tools emerging as a result (Li et al., 2009; Li and Durbin 2009). These, along with advancements in laboratory methods in extracting and preparing ancient DNA (Rohland & Hofreiter 2007a; Rohland & Hofreiter 2007b; Maricic et al., 2010; Dabney et al., 2013; Carpenter et al., 2013; Gansauge & Meyer 2013; Rohland et al., 2015), have paved the way for the reconstruction of entire ancient genomes (Green et al., 2010; Reich et al., 2010; Rasmussen et al., 2010; Rasmussen et al., 2011; Keller et al., 2012; Lazaridis et al., 2014; Olalde et al., 2014; Allentoft et al., 2015; Haak et al., 2015; Meyer et al., 2016).

Challenges of ancient DNA research

Although the reconstruction of whole ancient genomes is now possible, some challenges in obtaining and analysing ancient DNA persist. Throughout the life of an organism, the host's cellular metabolism as well as outside forces (e.g. UV radiation from the sun) introduce modifications to its DNA. However, in most cases the integrity of the host's DNA is efficiently restored through DNA repair mechanisms. Once an organism dies, however, the

repair mechanisms stop working and damage is allowed to accumulate within the host's DNA (Pääbo et al., 2004; Willerslev & Cooper 2005).

One of the main sources of post-mortem DNA damage is the hydrolytic deamination of bases, resulting in base misincorporations by polymerases during amplification (Lindahl 1993; Pääbo et al., 2004; Willerslev & Cooper 2005; Briggs et al., 2007; Dabney et al., 2013). Particularly susceptible bases are Cytosines, which after deamination become Uracils. These Uracils are subsequently paired with Adenines by polymerases during DNA amplification, which in the next cycle pair with Thymines instead of Cytosines, resulting in characteristic C-to-T or G-to-A 'mutations' (misincorporations) relative to a reference sequence. These misincorporations have been shown to occur more frequently than the error rate of DNA polymerases, and are absent in amplified modern DNA, making them a marker of DNA that has accumulated damage through time-dependant degradation and is ancient in origin (Gilbert et al., 2003a; Gilbert et al., 2003b; Binladen et al., 2006).

The consequences of these characteristic ancient DNA misincorporations are double-edged. Their presence in ancient DNA fragments can be used as evidence that the DNA is authentic and of ancient origin (Pääbo et al., 2004; Willerslev & Cooper 2005; Sawyer et al., 2012; Jónsson et al., 2013; Skoglund et al., 2014; Marciniak et al., 2015). Conversely, since most genetic analyses are sensitive to the number of differences between DNA sequences (mutations), the presence of these artificial 'mutations' can introduce erroneous and biased results and interpretations. As a result, an increasing number of studies treat the extracted ancient DNA with Uracil-DNA glycosylase to remove these artefacts from influencing downstream analyses (Rohland et al., 2015; Haak et al., 2015; Mathieson et al., 2015; Lazaridis et al., 2016; Olalde et al., 2018; Olalde et al., 2019). Alternatively, restricting analyses to sites which are unaffected by post-mortem DNA damage (e.g. transversions) can also be done, albeit at the cost of losing a sizable portion of potentially useful data (Prüfer et al., 2010; Fages et al., 2019; Günther & Nettelblad 2019; Der Sarkissian et al., 2020).

Another characteristic post-mortem modification of ancient DNA is its degradation into fragments of <500 base pairs in length (Pääbo et al., 2004; Willerslev & Cooper 2005; Marciniak et al., 2015). The phosphodiester bonds in the sugar-phosphate backbone of DNA are disrupted through hydrolytic cleavage and enzymatic reactions after cell death. This is complemented by hydrolytic cleavage of the glycosidic bonds which bind the bases to the DNA backbone, resulting in abasic sites which are susceptible to chemical reactions inducing further breaks in the DNA sugar-phosphate backbone (Dabney et al., 2013).

This large degree of degradation experienced by ancient DNA molecules over centuries and millennia results in them being present in miniscule concentrations compared to modern DNA, which is commonly found at high concentrations in many of our environments and workspaces. This large discrepancy in concentrations between ancient and modern DNA means experiments attempting to isolate low concentrations of ancient DNA can be easily swamped by sources of modern contamination, through people touching the bone, use of unsterilized equipment, or contaminated reagents (Cooper & Poinar 2000; Pääbo et al., 2004; Willerslev & Cooper 2005; Llamas et al., 2017). Computational methods for the detection of contaminated samples have been developed (Renaud et al., 2015; Nakatsuka et

al., 2020; Peyregne & Peter 2020) and are regularly used to identify problematic samples to be excluded from downstream analyses.

Many of the aforementioned advancements and characteristics of ancient DNA have been directly employed and considered in all four manuscripts presented in this thesis (manuscript A, B, C, and D). These four manuscripts build directly upon this previous work and the results presented would not be possible without the knowledge gained from the first three decades of ancient DNA research.

Ancient DNA and the prehistory of Western Eurasia

Although scientists had been working on ancient DNA for several decades, it has only been since ~2010 that the field has matured and contributed fully to the study of human history. Since 2010 (Green et al., 2010; Reich et al., 2010; Rasmussen et al., 2010; Rasmussen et al., 2011; Keller et al., 2012; Lazaridis et al., 2014; Olalde et al., 2014; Meyer et al., 2016), and at an increasing rate since 2015 (Haak et al., 2015; Allentoft et al., 2015; Mathieson et al., 2018; Olalde et al., 2018; Olalde et al., 2019; Mittnik et al., 2019; Narasimhan et al., 2019; Margaryan et al., 2020), data from thousands of ancient human genomes has been published, including studies which report more than 400 samples in a single publication (Narasimhan et al., 2019; Margaryan et al., 2020). This incredible growth in the number of samples has also been complemented by an expanding range, both geographic and temporal, of ancient DNA amenable to study (Meyer et al. 2014, Meyer et al., 2016; Lazaridis et al., 2016; van de Loosdrecht et al., 2018; van der Valk et al., 2021). The deluge of new data has provided many new insights, both expected and unexpected, into the history of our species. Since the four manuscripts (manuscript A, B, C, D) presented in this thesis build upon the results of many previous studies that have greatly increased our understanding and form the foundation of what is currently known, previous work will be summarised here.

Archaic humans (>40,000 years ago)

The sequencing of archaic genomes (Neanderthal and Denisovan) has provided important insights into timing of modern human origins, early modern human behaviour (e.g. interbreeding), genetic variation which defines our species, and how our species adapted to new environments (Sankararaman et al., 2014; Prüfer et al., 2017; Posth et al., 2017; Hajdinjak et al., 2018; Slon et al., 2018; Teixeira et al., 2021; Cooper & Stringer 2013). The introgression of Neanderthal ancestry in modern Eurasians is compatible with coming from one admixture event (although involved more than one Neanderthal individual) (Bergstrom et al., 2021) whilst the introgression of Denisovan DNA into eastern Eurasians and Oceanians was driven by at least two independent admixture events, involving deeply diverged Denisovan lineages (Browning et al., 2018). Evidence also exists of yet another, unknown ‘superarchaic’, hominin lineage which diverged from other humans ~2 million year ago contributing to the ancestors of Neanderthals and Denisovans hundreds of thousands of years earlier (Prüfer et al., 2014; Racimo et al., 2015; Rogers et al., 2020).

Research into the dynamics of archaic introgression into modern humans has shown that the majority of the introgressed DNA was deleterious to modern humans, with selection

acting to reduce the overall Neanderthal contribution through time (Fu et al., 2016). Large, functional parts of the human genome have been found to be 'Neanderthal deserts', containing no DNA inherited from Neanderthals and suggesting a degree of incompatibility between Neanderthals and modern humans (Sankararaman et al., 2014). This is particularly striking on the X chromosome, the majority of which is hemizygous in males and therefore particularly sensitive to deleterious variation.

However, some of the introgressed archaic variation was positively selected for and influences the biology of present-day populations. For example, genes affecting keratin filaments have shown to harbour high frequency of Neanderthal variants (Sankararaman et al., 2014). Similarly, introgressed archaic variants have been implicated in high altitude adaptation and susceptibility to Covid-19 (Huerta-Sanchez et al., 2014; Zeberg & Pääbo 2020).

Intriguingly, although all early modern humans studied to date show recent Neanderthal admixture, no late Neanderthals show modern human admixture (Hajdinjak et al., 2018), suggesting an asymmetric dynamic in the gene flow between these two groups.

Hunter-gatherers (~43,000-6,000 years ago)

Archaeogenetic studies of the earliest (>37,000-year-old) anatomically modern Europeans have shown that their genetic contribution to modern-day Europeans was minimal (Fu et al., 2014; Fu et al., 2015; Fu et al., 2016; Prüfer et al., 2021; Hajdinjak et al., 2021). This is often ascertained by investigating whether an ancient European shares more genetic affinity to later Europeans or East Asians (Fu et al., 2016). None of the earliest Europeans studied to date are genetically closer to later Europeans than they are to East Asians, suggesting they played a minimal role in the genetic origins of later Europeans. The ~37,000-year-old genome of Kostenki14 is the first to show more affinity to later Europeans than to East Asians, meaning the first ~8,000 years of human occupation in Europe left no major genetic trace in later Europeans (Fu et al., 2016). Interestingly, this may be related to the Campanian Ignimbrite volcanic eruption in Italy dated to shortly after 40,000 years ago which may have contributed to the demise of the earliest modern humans in Europe (Black et al., 2015, Giaccio et al., 2017).

Between 37,000 and 8,000 years before present, several genetic lineages have been detected in hunter-gatherers across Europe, likely associated with different cultural complexes (Fu et al., 2016). For example, the ancestry found in GoyetQ116-1, a ~35,000-year-old hunter-gatherer from Belgium, temporally associated with the Aurignacian technocomplex, is largely replaced by a new lineage associated with individuals of the Gravettian (34,000-26,000 years ago). However, the GoyetQ116-1 lineage did not go extinct as it is found to have contributed to later individuals of the Magdalenian technocomplex, dating 19,000-15,000 years ago (Fu et al., 2016; Villalba-Mouco et al., 2019). After 14,000 years ago, another genetic change is detected resulting in European hunter-gatherers carrying more ancestry related to present-day Near Easterners (Fu et al., 2016). This ancestry can be modelled as coming from a source related to an Epipalaeolithic hunter-gatherer from Anatolia (Feldman et al., 2019). As a result, the Palaeolithic and Mesolithic

history of Europe was dynamic and complex, with different hunter-gatherer ancestries appearing, disappearing and reappearing in different regions of Europe.

In general, Mesolithic Europe is made up of hunter-gatherers who can be broadly characterised as forming a genetic cline from the hunter-gatherers in the west (Western Hunter-Gatherers) to those in the east (Eastern Hunter-gatherers), with increasing affinity to an upper Palaeolithic Siberian individual (Raghavan et al., 2014) from west to east.

Neolithic revolution

The next major transition involved the origin and spread of agriculture, animal husbandry, and sedentism throughout western Eurasia. Much of the new data presented in this thesis (manuscript A, B, C, and D) comes from after the Neolithic revolution in Europe, a transition which facilitated larger population sizes and therefore more preserved skeletal material for study.

As with many major cultural transitions, it had long been debated to what extent agriculture spread through the transmission of ideas (cultural diffusion), a process which would result in a minimal demographic shift, or through the migration of farmers (demic diffusion), associated with a large demographic shift (Mortillet 1897; Richards et al., 2000; Achilli et al., 2004; Currat & Excoffier 2005).

It has been shown that the earliest farmers of the Levant, Anatolia and Western Iran descended largely from the pre-existing local hunter-gatherers in each region who adopted the sedentary farming lifestyle (Lazaridis et al., 2016; Feldman et al., 2019). These three groups (Levantine, Anatolian and Iranian farmers) were genetically highly differentiated, meaning the initial spread of farming around the fertile crescent was through cultural diffusion or independent innovation, without appreciable levels of gene flow. However, over ensuing millennia these three regions (Anatolia, Levant, Western Iran) became genetically connected and the genetic differentiation between these three regions reduced, likely through the exchange of both culture and people (Lazaridis et al., 2016; Skourtanioti et al., 2020).

The spread of farming to Europe was not a smooth wave of advance, but rather a process involving rapid expansions and extended periods of stagnation (Price & Bentley 2001; Shennan 2018). Although agriculture is attested in central Anatolia since ~8,300 BCE (e.g. Boncuklu Höyük) (Baird et al., 2012) and even earlier in Cyprus (>8,600 BCE) (Vigne et al., 2012), it would only spread further west around 7,000 BCE, reaching western Anatolia and the Aegean shortly thereafter (Schoop 2005; Brami 2015). Since the spread of agriculture to western Anatolia and the Aegean is considered to be part of the same wave of expansion (Shennan 2018), it is not surprising that ancient DNA studies of early Neolithic farmers from these two regions have shown them to be genetically almost indistinguishable (Mathieson et al., 2015; Hofmanová et al., 2016). Although no ancient DNA from hunter-gatherers of western Anatolia and the Aegean has been published, the strong genetic similarity between Aegean, west Anatolian and central Anatolian farmers is interpreted as evidence of a westward demic diffusion of central Anatolian farmers towards the Aegean.

Following its fast expansion from central Anatolia to the Aegean, the spread of agriculture halted for the next ~500 years (Shennan 2018). This halt is likely related to the difference in climate and environment between the Aegean and Balkan peninsula, meaning a period of adaptation to the newer conditions was necessary. The 8.2 kiloyear cooling event may also have played a role in slowing down the farmers' expansion. Then, starting around 6100 BCE, agricultural communities of the Starčevo culture spread widely and quickly throughout the Balkans (Weninger et al., 2014). Archaeologically, the Early Neolithic of the Balkans is similar in material culture and subsistence patters to that of the Aegean Early Neolithic. The archaeogenetic record of late hunter-gatherer and early agricultural groups is more complete in the Balkans than for the Aegean and Anatolia, allowing better insights into the spread of farming to this region. Early Neolithic farmers of the Starčevo culture were genetically distinct from the Mesolithic Iron Gates hunter-gatherers and genetically very similar to the famers of the Aegean and western Anatolia (Mathieson et al., 2018), suggesting agriculture spread through expanding farming groups who largely replaced pre-existing hunter-gatherers. However, there is also direct evidence for low levels of interbreeding between hunter-gatherers and incoming farmers, as one of the hunter-gatherers analysed (I5232) carries ~50% farming ancestry (Mathieson et al., 2018).

After spreading rapidly from the Aegean around 6,100 BCE and reaching Hungary by ~5,900 BCE, another period of stasis ensued (400-500 years) (Shennan 2018). The next pulse of the expansion of agriculture came with the appearance of the Linear Pottery culture (Linearbandkeramik – LBK) initially in western Hungary around 5,500 BCE (Bánffy et al., 2016; Jakucs et al., 2016). By ~5,300 the LBK culture had spread over 1,000 km, as far west as the Rhine river (Lefranc 2007; Denaire 2009; Denaire et al., 2011; Denaire et al. 2017) and Ukraine in the east. The homogeneity across much of its range, as seen in the pottery, architecture, settlement locations and soil preferences, attests to the fast spread of the LBK culture (Cladders 1997; Sommer 2001; Shennan 2018).

Archaeogenetic studies of people associated with the LBK culture have shown them to be genetically distinct from the pre-existing hunter-gatherers and almost indistinguishable from farmers of the Balkans, Aegean and Anatolia (Bramanti et al., 2009; Skoglund et al., 2012; Haak et al., 2015; Lipson et al., 2017). This suggests the fast spread of the LBK culture, much like the spread of farming from Anatolia to the Balkans, was a demic process involving the large-scale movement of people and minimal admixture with pre-existing hunter-gatherer groups.

Concurrent to the expansion of farming through the Balkans to central Europe along the Danube was a contemporaneous, maritime expansion of farming via the northern Mediterranean reaching Iberia and southern France about the same time as the appearance of LBK in central Europe (Zilhão 2001). Archaeogenetic studies have revealed that Early farmers of Iberia also carried a large degree of Anatolian Neolithic ancestry, revealing a similar pattern of demic diffusion as the catalyst for the spread of agriculture along the Mediterranean (Haak et al., 2015; Lipson et al., 2017; Villalba-Mouco et al., 2019). Early Neolithic farmers from southern France, however, appear unique in this regard by carrying 25-50% hunter-gatherer ancestry, suggesting more intense interaction and exchange with hunter-gatherers in this region in comparison to central Europe, Iberia and the Balkan peninsula (Rivollat et al., 2020).

Over a millennium after the appearance of agriculture in central Europe, it subsequently spread to Scandinavia and the Atlantic archipelago, arriving in both regions around 4,000 BCE (Skoglund et al., 2014; Brace et al., 2019). Here too, the arrival of the 'Neolithic package' was brought by individuals carrying mainly Anatolian Neolithic-like ancestry. The first farmers of Scandinavia show evidence of higher hunter-gatherer ancestry than those elsewhere, possibly resulting from interactions with the Neolithic hunter-gatherers of the Pitted Ware culture in the region. It appears as though this interaction may have been unidirectional, with no evidence of farming ancestry in the co-existing late hunter-gatherers (Skoglund et al., 2014).

Although the arrival of agriculture was driven by incoming farmers, in many parts of Europe the pre-existing hunter-gatherers did not go extinct. Instead, they likely co-existed with farmers for the next 2-3 millennia. Although they left little traces in the archaeological record, hunter-gatherers left evidence of their co-existence in the genomes of middle and late Neolithic farmers. In most regions of Europe, the proportion of hunter-gatherer ancestry in farmers increases ~3-4-fold in the two millennia after the appearance of early Neolithic farmers (Haak et al., 2015; Lipson et al., 2017; Mathieson et al., 2018). Although the general trend of increasing hunter-gatherer ancestry in farmers during the Neolithic happened in several regions (e.g. Iberia, Germany, Hungary), the type of hunter-gatherer ancestry incorporated was not the same, but rather the local hunter-gatherer ancestry present in each region (Lipson et al., 2017). In addition to this indirect evidence of the late persistence of hunter-gatherers, several sites have revealed direct evidence of hunter-gatherer genetic profiles well into the 4th millennium BCE (e.g. Blatterhöhle, Tangermünde) (Lipson et al., 2017; Rivollat et al., 2020).

The increase in biological interaction between farmer and hunter-gatherer groups through time may have been aided by later farming groups moving away from predominantly exploiting loess sediments, making them more readily come into contact with hunter-gatherer groups (Shennan 2018). Although the social process by which hunter-gatherer ancestry increased in farming groups across Europe is poorly understood, the general consensus is that, after the introduction of farming to Europe, little demographic change occurred until ~3,000 BCE.

"Steppe"-related ancestry

The third millennium BCE has been shown to have been a highly dynamic period in European prehistory, with major changes in both the archaeological and archaeogenetic records (Haak et al., 2015; Allentoft et al., 2015; Olalde et al., 2018). As a result, much of manuscript A will focus on this period from a population genetic and social perspective, which will be complemented by findings from manuscript C.

The Corded Ware (CW) cultural complex appeared in central, northern and north-eastern Europe around 2,900 BCE amidst preceding cultures which showed low incidence of single graves and no gender differentiation within graves. This changed profoundly with the CW culture, which brought three major changes in mortuary practices. First was the significant increase in single graves. This sharp increase in single graves likely indicates a major

ideological change within CW society, during which the individual appeared to become more significant and emphasised in the burial ritual. The second major change was the appearance, for the first time, of a relatively strict gender differentiation in the burial custom. Males and females were buried with their bodies in opposite orientations, suggesting different genders being viewed differently or having differentiated roles in CW society. The third major change evident in CW is the rise of a male warrior symbolism, as seen through the increased incidence of battle axes found in male graves. Taken together, these changes represent a novel and unique cultural complex whose origins have been debated (Kristiansen et al., 2017; Furholt 2021).

Building upon breakthroughs from ancient mitochondrial variation (Brandt et al., 2013), large advances in our understanding of the origin of the CW culture came in 2015 with the first ever analyses of genome-wide data from CW individuals (Haak et al., 2015; Allentoft et al., 2015). These studies showed that a large fraction (~75%) of the ancestry found in the CW individuals analysed from Germany and Poland was never before found in Mesolithic or Neolithic Europeans. Instead, this ancestry was similar to individuals ascribed to the archaeological pit grave (Yamnaya) culture (~3300-2600 BCE) of the Pontic-Caspian steppe (hence dubbed "Yamnaya" or "steppe" ancestry). Both studies concluded that the CW culture represents a "large-scale" or "massive" westward migration of Yamnaya-related people into central Europe, who themselves were found to be an approximately equal mixture of people related to eastern European hunter-gatherers and hunter-gatherers from the Caucasus (Haak et al., 2015; Jones et al., 2015). Subsequent studies have analysed additional CW individuals and come to similar conclusions (Malmström et al., 2019; Linderholm et al., 2020; Furtwängler et al., 2020; Saag et al., 2021). Some studies have suggested this to have been a male-biased migration (Goldberg et al., 2017; Mittnik et al., 2019), while others have disputed that (Lazaridis & Reich 2017).

In contrast to the spread of farming ancestry, Yamnaya-related "steppe" ancestry spread rapidly throughout Europe, reaching Ireland, Iberia, and various Mediterranean islands by the end of the 3rd millennium BCE (Cassidy et al., 2016; Martiniano et al., 2017; Olalde et al., 2018; Olalde et al., 2019; Marcus et al., 2020; Fernandes et al., 2020). "Steppe"-related ancestry has been found in the majority of CW and later dated individuals, attesting to the fast spread and continent-wide impact of this ancestry in post-CW people, including modern Europeans. This impact is well exemplified by the 90% genetic turnover that accompanied the appearance of the Bell Beaker (BB) culture in modern-day Britain as well as the "almost complete" turnover in Y-chromosome lineages in Early Bronze Age Iberia (Olalde et al., 2018; Olalde et al., 2019).

The amount and speed of genetic change uncovered by recent archaeogenetic studies, especially in the third millennium BCE, has surprised many archaeologists. Some archaeologists have criticised the recent interpretations, citing concerns over sampling size/bias and the overly simplistic narratives deduced therefrom (Vander Linden 2016; Heyd 2017; Klejn 2017; Klejn 2018; Furholt 2018; Furholt 2019a; Furholt 2019b; Furholt 2021). However, sample sizes have increased dramatically since 2015 and largely confirmed the previous patterns in the geographic and temporal distribution of ancient European genetic variation (Olalde et al., 2018; Olalde et al., 2019; Malmstrom et al., 2019; Mittnik et al., 2019; Linderholm et al., 2020; Furtwängler et al., 2020; Saag et al., 2021).

Currently, no clear explanations exist to account for the archaeogenetic observations of large-scale and continent-wide genetic turnovers in third millennium BCE Europe. One potentially promising observation may be the evidence for population declines across Europe in the late fourth millennium BCE, a few centuries prior to the largescale expansion of "steppe" ancestry (Shennan et al., 2013). This decline in population density in central Europe, as inferred from the low incidence of archaeological finds and human remains, may have facilitated population expansions from the east by opening lands with less competition than in the migrants' original homelands. This, coupled with potentially new technologies known to have existed in the east (e.g. domesticated horses, wagons) may have allowed migrants from the east to successfully expand their geographic range (Anthony 2010). Interestingly, the earliest evidence for *Yersinia pestis*, the causative bacterium of plague, is also from early third millennium BCE Europe (Rascovan et al., 2019). This bacterium is known to have caused some of the largest pandemics in human history (Bos et al., 2011; Keller et al., 2019; Spyrou et al., 2019) and its presence during the highly dynamic third millennium BCE may help explain some of the large-scale migrations which took place.

By the end of the third millennium BCE, most regions of Europe had received "steppe" ancestry gene flow, the majority of which was spread by expanding CW and, later, BB groups. It is with the arrival of this ancestry component, in addition to the hunter-gatherer and farming ancestry already present millennia earlier, that the majority of the modern European gene pool had formed. The majority of modern European ancestry originates from the mixture of these three ancestry components, with different regions having different proportions of each component (Haak et al., 2015).

Since languages can be spread by migrating people, the discovery of a large, continent-wide demographic event in the third millennium BCE has been interpreted as support for, at least some, Indo-European languages originating on the Pontic-Caspian steppe and spreading around Eurasia within the last 6,000 years (Haak et al., 2015; Allentoft et al., 2015; Kristiansen et al., 2017). This is supported by the finding that Yamnaya-like "steppe" ancestry also spread east, into central Asia and the subcontinent, both regions known to have been inhabited by Indo-European speakers. However, no "steppe" ancestry has so far been found in Anatolia, an important region which attests to the earliest branching Indo-European languages.

Current shortfalls

Although our understanding of European prehistory, and the third millennium BCE in particular, has improved greatly in the last decade, many open questions remain. Archaeogenetic studies have reported large-scale shifts in genetic diversity, however little is known about the underlying mechanisms and reasons behind these observed patterns. This is partly due to the low density, continent-wide sample strategy employed by most of the studies upon which our understanding is built on (Haak et al., 2015; Allentoft et al., 2015; Mathieson et al., 2015; Olalde et al., 2018; Mathieson et al., 2018). Although such a sampling strategy has proven fruitful in mapping out a scaffold for how genetic diversity has changed through time, many gaps remain to be filled with important insights into how geographically and temporally overlapping cultural groups influenced and gave rise to one

another. The primary aim and unifying theme of the manuscripts presented in this thesis (manuscript A, B, C, and D) is to address the current shortfalls mainly through a detailed, well-contextualised, and densely sampled dataset of central Europe. The densely settled and archaeologically well studied nature of prehistoric Bohemia allows for the assembling of a high-resolution dataset encompassing many of the cultural transitions seen in Europe more broadly (manuscript A and B), something which is lacking in the current archaeogenetic dataset.

For example, currently the largest sample size of CW individuals has been studied from Esperstedt (Saxony-Anhalt, Germany) (Haak et al., 2015; Mathieson et al., 2015) and southeast Poland (~15 from each region) (Linderholm et al., 2020). However, the CW individuals published from both regions are almost exclusively from the late phase of CW (after 2600 BCE), dating to more than 300 years after the appearance of the CW culture in the region and offering limited insights into the origin of the earliest CW migrants. In addition, from both regions there is currently a lack of genetic data from the transitional period during which both the last pre-CW and earliest CW people co-existed (3000-2600 BCE), resulting in limited insights into the interactions between CW and pre-CW groups. Finally, both regions are undersampled for Early Bronze Age individuals (~7 from Saxony-Anhalt, 0 from southeast Poland), meaning we have limited knowledge about the origin and spread of the Early Bronze Age Únětice culture. The newly constructed dataset from Bohemia presented in manuscripts A and B from this thesis directly address such shortfalls by filling in these temporal sampling gaps and providing important added resolution to the cultural transitions therein.

Currently, the best studied regions of Europe from an archaeogenetic perspective are Iberia (n=529) and the Atlantic archipelago (n=449) (Olalde et al., 2014; Olalde et al., 2015; Günther et al., 2015; Mathieson et al., 2015; Fu et al., 2016; Schiffels et al., 2016; Cassidy et al., 2016; Martiniano et al., 2016; Lipson et al., 2017; Olalde et al., 2018; Valdiosera et al., 2018; Sanchez-Quinto et al., 2019; Olalde et al., 2019; Brace et al., 2019; Fernandes et al., 2020; Cassidy et al., 2020; Margaryan et al., 2020). Although these data have provided important insights into the population histories of their respective regions, both regions are peripheral cul-de-sacs of Europe located on the opposite side of the continent from the entry points of the major migrations which shaped European genetic diversity, specifically southeast Europe (“farmer” ancestry) and eastern Europe (“steppe” ancestry). As a result, Iberia and Britain give us important insights into the speed at which these ancestries spread throughout Europe and reached its peripheries, but less information on the origins and mechanisms of dispersal throughout Europe.

Important in understanding the cultural and genetic transitions of the European third millennium BCE are regions which were settled by all the major archaeological cultures therefrom, including Globular Amphora, Corded Ware, Bell Beaker and Únětice. Currently, no such region in Europe has been studied from an archaeogenetic perspective. As a result, despite the increasing Europe-wide number of ancient genomes available today, the cultural transitions in prehistoric, especially third millennium BCE, Europe remain poorly understood.

The first unresolved question concerns the genetic and geographic origin of CW individuals, and their relationship to Yamnaya individuals of the Pontic-Caspian steppe. Although the consensus among geneticists is that CW individuals result from a “massive” and “fast” westward migration of Yamnaya individuals (Haak et al., 2015; Allentoft et al., 2015), several observations are not compatible with this scenario. First, R1a, the dominant Y chromosome lineage found in >70% of CW males, has so far never been found in Yamnaya males. Although this does not exclude R1a from being found in Yamnaya males in the future, it shows that the sampled Yamnaya males were not the paternal genealogical ancestors of the sampled CW males.

In addition, if the origin of CW is to be explained by a “massive” and “fast” westward migration of Yamnaya, we may expect to find some CW associated individuals who retain a 100% Yamnaya genetic profile. However, this is not the case as can be seen on PCA where there is no overlap in the distribution of published CW and Yamnaya individuals, even for CW individuals who are geographically close to the Pontic-Caspian steppe (i.e. Fatyanovo individuals from western Russia (Saag et al., 2021)). These observations, taken together, suggest that the currently sampled Yamnaya are not the direct source of “steppe” ancestry in CW individuals and argue against a “massive” and “fast” migration of Yamnaya individuals from the Pontic-Caspian steppe as the origin of CW. Consequently, the question of the geographic origins of CW, their relationship to Yamnaya individuals, as well as the nature of their migration (e.g. sex bias, interactions with other cultural groups) remain open.

From an archaeological perspective, the earliest Bell Beakers appeared in Portugal around ~2800 BCE, after which they spread rapidly throughout many regions of western Europe. The origin of the BB culture has been shown to have been different in Iberia and central Europe (Olalde et al., 2018). In contrast to Iberian, central European BB individuals carry “steppe” ancestry supposedly similar to that of Yamnaya individuals of the Pontic-Caspian steppe, although on average less than is found in CW individuals (Haak et al., 2015; Mathieson et al., 2015). However, modelling central European Bell Beaker individuals as three-way mixtures of western hunter-gatherers, Anatolia Neolithic and Yamnaya individuals fails (personal reanalysis of published data), suggesting that BB individuals carry additional ancestry not represented within these three sources. Further, BB males carry predominantly Y chromosome R1b-P312, a lineage so far not found in CW or Yamnaya males. As a result, it is currently not possible to link Yamnaya, CW and BB groups as being direct paternal sources for one another, despite their sharing of “steppe” ancestry and their partial temporal overlap.

Finally, little is known about the origin of the Early Bronze Age in central Europe, including individuals of the Únětice culture. The few Únětice individuals published so far have all shown the presence of “steppe” ancestry, however their genetic make-up relative to the immediately preceding late BB individuals has not been evaluated. The current Y-chromosome data available from Únětice males suggests a markedly different frequency of R1b-P312 compared to the preceding BB males, suggesting significant demographic change accompanying the spread of EBA cultures, as has been shown in Iberia (Olalde et al., 2019). However, data is lacking from the transitional period between central European BB and EBA cultures (~2300-2100 BCE) precluding our understanding of this cultural transition. This is directly addressed in manuscript A, which significantly expands the sample size from this

transitional period, allowing novel and important insights into the origin of the Early Bronze Age in central Europe. Although it has been hypothesised that Early Bronze Age Europe was the first truly globalised period with complex social organisation (Kristiansen & Larsson 2005; Meller 2017; Meller 2019), comparatively little is also known about their social structures and kinship systems (Mittnik et al., 2019). This shortfall is addressed in manuscript B, with a detailed investigation of an Early Bronze Age Únětice cemetery from eastern Bohemia.

Ancient DNA and human pathogens

The Neolithic revolution had a profound impact on the lifestyle of humans. Firstly, it brought people in closer and more frequent contact with a range of animal species, including goats, sheep, cattle and pigs. Secondly, it facilitated much larger population sizes, resulting in the formation of new proto-cities such as at Çatalhöyük in southern Anatolia. These two factors exposed farming communities to a range of new pathogens which could potentially infect them through zoonosis (Boyden 1970; Fenner 1970; Cockburn 1971; Armelagos & McCardle 1975; Barrett et al., 1998; Bos et al., 2014; Mühlemann et al., 2018a, Krause-Kyora et al., 2018). The increase in trade throughout the Neolithic and Bronze Age also paved the way for potential outbreaks of disease to be carried much further than the community in which they arose, potentially affecting wider regions or whole continents.

Prior to archaeogenetic methods, the study of ancient diseases largely relied on three sources of information, namely the analysis of modern disease strains, the phenotypic manifestations of lesions and disease-causing agents in archaeological remains, and the historical texts describing aspects of past diseases (Benedictow 2004; Cunha 2004). However, all three sources have drawbacks, with modern variation being blind to strains that have gone extinct, the majority of acute infections failing to leave traces on bones or being ambiguous with respect to the causative disease (Ortner 2003), and most societies of the human past leaving no historical texts.

Archaeogenetic analyses of ancient pathogens have added both to our understanding of the past, by being able to directly evaluate the disease-causing agents of past pandemics (Bos et al., 2011; Schuenemann et al., 2018), as well as to our understanding of pathogen evolution in general, by understanding the phylogeographic relationships in ancient pathogen diversity, identification of extinct strains (Rasmussen et al., 2015; Andrades Valtueña et al., 2017), understanding how quickly they mutate, and how they adapt and evolve through time (Key et al., 2020).

Early studies of ancient pathogens experienced similar difficulties to those aforementioned in ancient DNA research in general, with PCR-based methods providing limited data and resolution (Spigelman & Lemma 1993; Salo et al., 1994; Arriaza et al., 1995; Drancourt et al., 1998; Zink et al., 2001). However, soon after the breakthrough publications of the Neanderthal (Green et al., 2010) and Denisovan (Reich et al., 2010) genomes came the reconstruction of the first ancient bacterium, the *Yersinia pestis* strain responsible for the Black Death (Bos et al., 2011). This genome appeared genetically similar to the most recent common ancestor of modern-day *Yersinia pestis* strains, a finding which was interpreted as suggesting that it was the environmental conditions in which the Black Death strain found

itself, and not something specific about the strain itself, which caused the most fatal pandemic in human history.

Yersinia pestis remains the best studied pathogen to date, with several factors making it particularly interesting to study. The first is its inherent characteristics, including its stable double-stranded DNA structure, manageable genome size, and blood-borne nature making it amenable to detection and investigation (Parkhill et al., 2001). From a human health perspective, its implication in some of the deadliest pandemics in human history (Bos et al., 2011; Wagner et al., 2014) has made its biology and evolution important to understand. Additionally, its finding as early as in Late Neolithic northern Europe (Rascovan et al., 2019), a very dynamic period in European history where fast, continent-wide migrations have been attested (Haak et al., 2015; Allentoft et al., 2015; Olalde et al., 2018), has prompted speculation into its role in the speed and wide-spread movement of people in this period, perhaps through driving a depopulation of central Europe in the late 4th millennium BCE (Hinz et al., 2012; Shennan et al., 2013; Kristiansen et al., 2017).

However, it has also been shown that other pathogens, such as Hepatitis B virus (HBV) and parvovirus B19V, were also present during this time (Mühlemann et al., 2018a; Krause-Kyora et al., 2018; Mühlemann et al. 2018b). In contrast to the spread of human genes, pathogens can spread horizontally through mere contact with infected hosts. As a result, understanding the phylogeographic distribution of ancient pathogens has the potential to inform about past migratory events or spheres of interaction (Andrades Valtueña et al., 2017), even in cases where minimal genetic exchange or mixture between human groups occurred. Ancient pathogen data provides an added perspective and layer of complexity to our understanding, and should be considered when interpreting the past. Manuscript C of this thesis will present the largest study of ancient HBV to date, providing new insights into its origin, evolution, and spread.

Ancient DNA and social anthropology

Perhaps the hitherto least explored topic to which archaeogenetic research can contribute is the understanding of the functioning of ancient societies through detailed analyses of social processes and kinship systems. Although insights into social aspects of ancient societies can be gleaned from archaeology (e.g. collective burials in pre-Corded Ware northern Europe, rise of male warriors in Corded Ware burials, segregation of male and female roles in CW and BB societies due to sex-differentiated burials) (Vander Linden 2007), little is known about patterns of biological relationship in ancient societies, and how kinship was organised and reflected in aspects of mortuary archaeology (e.g. grave goods, inheritance) and social status. Some studies have already shown that continent-wide and sudden turnovers in Y-chromosomal diversity (Zeng et al., 2018; Olalde et al., 2018; Olalde et al., 2019) occurred in 3rd millennium BCE Europe, likely implying a different social organisation and/or power dynamic between incoming migrants and locals. However, little is known about the underlying process of this change, especially at the level of local and neighbouring communities and regions.

Patterns of biological kinship can be inferred from ancient DNA, and when combined with dense sampling of whole cemeteries, mortuary archaeology and other scientific analyses

(e.g. stable isotopes), insights into the functioning of ancient societies can be drawn (Haak et al., 2008; Knipper et al., 2017; Kennett et al., 2017; Mittnik et al., 2019; Sjögren et al., 2020). Dense analysis of ancient cemeteries allows reconstruction of pedigrees, patterns of social behaviour, individual mobility, and aspects of inheritance and social status to be inferred. With such studies already underway in the Lech valley of Bavaria (Knipper et al., 2017; Mittnik et al., 2019), the detailed investigation of a contemporaneous EBA cemetery in Mikulovice (eastern Bohemia) presented in manuscript B will allow for direct comparisons between EBA social organisation in different regions of central Europe.

A note on archaeological "cultures"

Some of the recent archaeogenetic publications have been criticised by archaeologists on the grounds of misrepresenting archaeological "cultures" as being homogenous social units (Vander Linden 2016; Heyd 2017; Furholt 2018), perhaps something akin to ethnic groups, and bringing back long-outdated concepts of culture-historical archaeological thinking (Shennan 1976; Clarke 1978; Furholt 2014). This has been deemed problematic partly due to the past association of such thinking with nationalistic political agendas, a reason for why such thinking was quickly superseded after the second world war in favour of other explanations of shared material culture (Kossinna 1911; Anthony 1990). In addition, new research has shown that the seemingly homogeneous archaeological "cultures", which were claimed to be uniform across large distances, vary substantially within their geographic range (Furholt 2014).

The heterogeneity in the archaeological record, in addition to the small sample sizes in early archaeogenetic studies (e.g. 4 Corded Ware individuals in Haak et al., 2015) was indeed a cause for concern, especially when broad, continent-wide sweeping statements and conclusions were made about "massive migrations" based on such small sample sizes. However, since the publication of these studies, much more data has become available which has largely confirmed the original findings of two large genetic turnovers in Europe associated with the arrival of agriculture and appearance of the CW culture. As a result, it can be argued that the studies of 2015 (Haak et al., 2015; Allentoft et al., 2015) provided a broad framework outlining the major genetic turnovers in Neolithic Europe, upon which more recent studies have built a more nuanced understanding (Mathieson et al., 2018; Furtwängler et al., 2020; Linderholm et al., 2020; Egfjord et al., 2021). Much like geographic heterogeneity in material culture, it is likely that future archaeogenetic studies will reveal regional heterogeneity in population history and social processes, as has been found in Switzerland, with the existence of individuals lacking "steppe" ancestry well into the 2nd millennium BCE (Furtwängler et al., 2020).

From the perspective of this thesis, manuscript A deals with archaeogenetic data from the northern part of Bohemia, while manuscript B deals with data from a single site, also situated in the northern part of Bohemia. By focussing on such confined geographic region as is Bohemia, the argument of heterogeneity in material culture across space is largely nullified, at the expense of the insights being confined largely to Bohemia. Since the resolution of most genetic analyses (e.g. f-statistics, *qpWave*, *qpAdm*) is increased by grouping individuals, individuals are grouped based on (where possible) radiocarbon dates and association with archaeological cultures. Association to an archaeological culture is made based on a combination of criteria, including associated grave goods, body orientation, and knowledge of the local chronology and which archaeological cultures are

typically found at the time in question. We acknowledge that the concept of "archaeological culture" may be problematic and the resulting groups may not reflect cohesive social units, however archaeological units of classification (i.e. cultures, phenomena, complexes, horizons) still provide a seemingly logical way of grouping samples which come from similar historical (geographic, temporal, archaeological) contexts.

Manuscript A offers a unique insight into how genetic diversity changes within an "archaeological culture" through time. Through dense sampling and fine temporal resolution (many radiocarbon dates), archaeological units of classification of the 3rd millennium BCE (CW, BB, Únětice) are grouped into early and late phases, offering a glimpse into how they change through time. Manuscript A shows that these units of classification are not genetically homogeneous throughout their time of existence.

Archaeological background to Bohemia

In order to address some of the aforementioned shortfalls in our current understanding of Neolithic and Early Bronze Age Europe, this thesis will focus on archaeogenetic data from Bohemia (Fig. 1). Located in the heart of Europe and forming the western part of today's Czech Republic, the fertile lowlands of the northern part of Bohemia have attracted many societies from different archaeological cultures and time periods. The presence of the important Elbe (east-west) and Vltava (north-south) rivers (Fig. 2) have not only provided a stable source of water, but also likely acted as ancient pathways of movement, communication and exchange. Complemented by a long tradition of archaeological research (Ryzner 1880a; Ryzner 1880b) and conditions (e.g. temperate climate) which facilitate the preservation of ancient DNA, Bohemia proves an important and attractive region for a detailed archaeogenetic investigation.

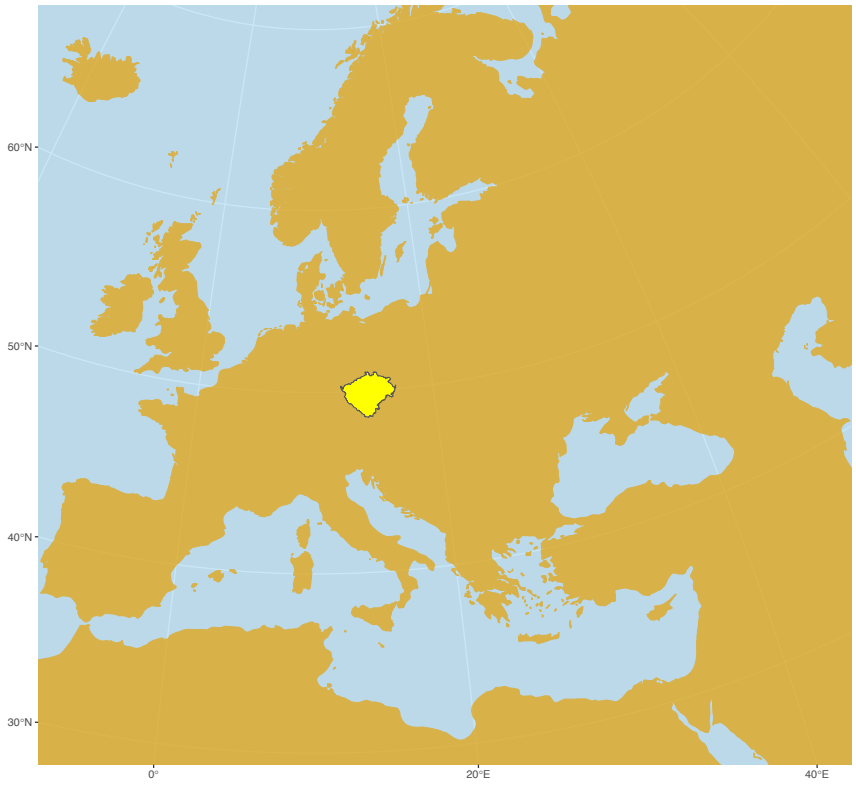

Fig. 1. Bohemia's central geographic location (yellow) contributes to its importance in understand the population history of Europe.

Throughout prehistory, most human occupation has been focussed around the lowlands of the northern part of Bohemia, the basins of the three main rivers running through the region, namely the Elbe, lower Vltava and Ohře rivers. The southern and southwestern parts of Bohemia are of higher elevation and more forested, meaning they were less attractive for farming communities, and only became more densely and continuously settled since the Early Bronze Age. It is thought that hunter-gatherers may have persisted in the southern, forested regions of Bohemia during the Neolithic and Eneolithic, but direct archaeological evidence of their presence is lacking (Vencl 1982).

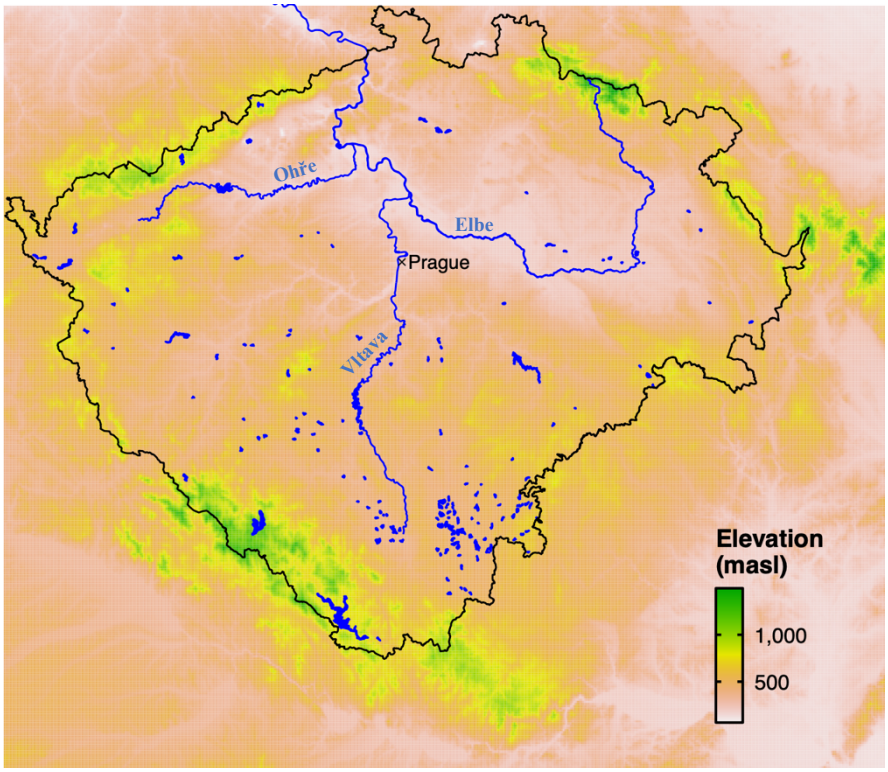

Fig. 2. Map of Bohemia showing lowlands of the north and major bodies of water, including Elbe, Vltava and Ohře rivers.

Neolithic (~5,400-4,400 BCE)

Much like the rest of central Europe, the Neolithic in Bohemia began around 5,500-5,400 BCE with the arrival of the Linearbandkeramik (LBK) culture and the accompanying "Neolithic package" from the southeast. This shift represented a fundamental change in the dominant subsistence strategy in the region, changing from reliance on hunting and gathering of wild species to the raising of domesticated animals (e.g. cattle, pig, sheep, goat), grains, and legumes (Vencl 1982). This was complemented by the production of ceramics, textiles, and extensive exploitation of woodlands for timber in longhouses, enclosures, and wells (i.e. settlement infrastructure). Although almost all of the wood used in tools and LBK longhouses has not survived until today, the subterranean nature of wells has facilitated the preservation of the wood used in some instances. The preserved wood can sometimes be accurately dated using tree-ring chronologies (Tegel et al., 2012). Among the earliest dendrochronologically dated LBK sites in Europe is Mohelnice, in neighbouring

Moravia, from where a wooden well has been dated to 5,450 BCE (Pavlů 2005). Specifically from Bohemia, one of the most important LBK sites, Bylany, records occupation starting ~5,400 BCE (Pavlů & Zápotocká 2007).

The Neolithic in Bohemia is divided into the Early Neolithic, lasting from ~5,400-5,000 BCE and associated with the LBK culture, and the Late Neolithic, lasting from ~5,000-4,400 BCE and associated with the Stichbandkeramik (STK) culture. It is thought that the STK derives directly from the LBK without major external influences (Pavlů & Zápotocká 2013). The Bohemian Neolithic archaeological record contains more evidence of settlements than graves throughout the entire Neolithic (~5,500-4,400 BCE), with sites containing graves constituting less than 10% of all Neolithic sites. The most common archaeologically attested mortuary practice during the Neolithic was single grave inhumations, with cremations also known but present in the minority of cases. However, it is also thought to be highly likely that other, archaeologically undetected, mortuary practices existed, including the scattering and deposition of human skeletal remains in areas surrounding the settlements (Zápotocká 1998). As across their entire distribution, LBK settlements in Bohemia are commonly found on fertile loess soils, in close proximity to a water source, usually in the form of rivers and creeks. Settlement organisation usually revolved around the LBK longhouses, which are architecturally similar across the entire LBK geographic range (Shennan 2018).

Eneolithic (~4,400-2,200 BCE)

The Neolithic in Bohemia is followed by the Eneolithic (~4,400-2,200 BCE), a period contemporaneous with the “Jungneolithikum” (young Neolithic), “Spätneolithikum” (late Neolithic) and “Endneolithikum” (final Neolithic) of the neighbouring German chronology (Lüning 1996). The Eneolithic is defined by a suite of technological innovations, the main one being the presence of copper artefacts. However, this definition has fallen out of favour since copper is rare (e.g. in CW) or absent (e.g. Globular Amphora) in some Eneolithic cultural groups (Neustupný et al., 2013). In addition to the presence of copper, the Eneolithic in Bohemia is also distinguished from Neolithic by the use of ploughs which were pulled by animals (e.g. oxen). This was complemented by the disappearance of large Neolithic villages in favour of smaller, lower-density, settlements, as well as the increased incidence of weapons and fortifications. It is believed that Eneolithic communities were small, possibly comprising of about three families (Neustupný et al., 2013). Although settlements were smaller in the Eneolithic, they may have been occupied for longer periods of time, since ploughing required the investment of resources and maintenance of fields for several generations.

The emergence of the Lengyel culture (~4,400-4,200 BCE) in Bohemia marked the beginning of the Eneolithic (proto-Eneolithic, ~4,400-3,900 BCE) and is thought to have been introduced through an influence from the southeast (Pavlů & Zápotocká 2013, Neustupný et al., 2013). The subsequent Jordanow culture (~4,200-3,900) is thought to be largely the continuation of the Lengyel culture, with later phases of the Jordanow culture also showing influences from the Michelsberg culture from further west (Neustupný 2013b, Zápotocký 2013).

The Early Eneolithic (~3,900-3,400 BCE) in Bohemia is represented by the Funnelbeaker culture, which is also found in Poland, northern Germany, the Netherlands and southern Scandinavia. Typical at the northern distribution of the Funnelbeaker culture were burials in collective megalithic tombs, as was also the case for most western European cultural groups at the time. However, in Bohemia this is not the case, with most (>100) Funnelbeaker graves being single inhumation burials, while several dozen burials in settlement features are also known. From the mortuary perspective, the Funnelbeaker burial tradition in Bohemia is similar to that of the Neolithic LBK and STK cultures (Neustupný et al., 2013).

The Middle Eneolithic (~3400-2800 BCE) in Bohemia is characterised by Baden and post-Baden associated cultures. The origin of the Baden culture is thought to have ties to its development in the Carpathian basin (Zápotocký 2000), eventually giving rise to the post-Baden (~3100-2800 BCE) Řivnáč (central and northwestern Bohemia), Bošáca (southwestern Slovakia, Moravia and eastern Bohemia), Cham (southwestern Bohemia and Bavaria), and Jevišovice (southern Moravia) cultures. Inhumations from this period are rare (~20 known burials in Bohemia) and the few human remains that are known come from sunken settlement features (e.g. pits, huts) (Neustupný et al., 2013). This lack of burials from the Middle Eneolithic, a feature of this period known also from neighbouring regions, has been interpreted as either being a sign of a regional population decline (Shennan et al., 2013) in the late 4th millennium BCE or a change in mortuary practices to archaeologically unidentifiable ways of disposing of the deceased. The recent finding of *Yersinia pestis*, the causative agent of the black death, in the remains of a Late Neolithic (~2900 BCE) individual from Sweden's Funnelbeaker culture (Rascovan et al., 2019) has raised the possibility of a similar pandemic driving widespread depopulation. The end of the Middle Eneolithic (~3000-2800 BCE) sees the appearance of the Globular Amphora culture, regarded by most archaeologists as representing newcomers to Bohemia from north/northeast (Dobeš 2013, Neustupný 1982).

The Late Eneolithic (~2900-2200) in Bohemia is characterised by the CW (~2900-2400) and BB (~2500-2200) cultural phenomena. Both have several unique features which are not seen in previous times. One is their incredibly large geographic distribution, with both cultures stretching over vast regions of the European continent. Another is their relatively strict gender differentiation in their burial ritual, with males and females buried in opposing body orientation (Furholt 2021). Yet another is the lack of settlements which can be attributed to these cultures, which some archaeologists have interpreted as being evidence that these groups were more mobile (or associated with more pastoralist way of life) compared to previous cultures. The origin of CW and BB, as well as how they achieved their continent-wide distribution, has long been debated among archaeologists, with a range of hypotheses, from local origins and cultural diffusion to large-scale migrations, having been put forth (Buchvaldek 1980, Turek 2013, Borkovskyj 1932, Neustupný 2013a, Neustupný 1969, Vencl 1994, Moucha 1978, Neustupný 1976).

Early Bronze Age (~2200-1700 BCE)

The Early Bronze Age in Bohemia is represented by the Únětice culture (~2200-1700 BCE), commonly separated into the early (all pre-classical phases, pre-2000 BCE) and late (classical and post-classical, post-2000/1950 BCE) phase (Jiráň et al., 2013, Moucha 1963, Bartelheim

1998). Based on similarities in some aspects of the burial practices and between certain subsets of BB and Únětice pottery, it is generally considered that the Únětice largely derived from the preceding local BB groups, with influences also potentially stemming from the Carpathian basin or southeast in general. The classical phase of the Únětice culture is characterised by large flat-grave cemeteries with dozens of wealthily equipped crouched burials, often placed under sophisticated stone constructions, and containing large amount of cast bronzes (pins in their hundreds, bracelets, daggers or axes), golden earrings, and necklaces composed of tens to hundreds amber beads and other exotic items. Large bronze hoards are also typical as well as the so called Únětice eyelet-pins and the Únětice cups in their hundreds (Moucha 2005, Ernée 2012, Ernée 2013, Ernée 2016, Ernée 2017, Limburský et al., 2018, Ernée et al., 2020).

The central location, in addition to its rich archaeological record makes Bohemia an interesting and important region to study in order to understand the cultural, social and genetic transitions throughout the Neolithic and Early Bronze Age central Europe. In addition to the rich archaeology of the region is the long tradition of archaeological inquiry (Ryzner 1880a; Ryzner 1880b), meaning many of the cultures found in Bohemia have been well studied, documented and contextualised, with robust chronologies and historical and anthropological hypotheses to test for the first time using ancient DNA data.

2. Aims of the thesis

The overarching aim of this thesis is to achieve a better understanding of the population history, social structure, and intergroup exchange in Neolithic to Bronze Age Europe. To achieve this, the thesis will focus on new archaeogenetic data generated from Bohemia, a key region in central Europe which attracted members of many different cultural groups throughout prehistory. This, coupled with the favourable environmental conditions for ancient DNA preservation renders Bohemia an attractive region for a detailed archaeogenetic study.

The aims are achieved primarily by increasing the resolution of the available archaeogenetic record in central Europe both vertically, in a transect through time in Bohemia, as well as a frozen in time large-scale intra-site study focussed on the Early Bronze Age cemetery in Mikulovice. Generating new genetic data from 283 individuals, in addition to the 65 previously published individuals, from the northern part of Bohemia makes this region currently one of the densest regions sampled for ancient human DNA in the world. In addition to the expanded sample size, increased resolution is also achieved through the development of a new capture technique for a more detailed understanding of Y-chromosome diversity in ancient males.

The insights gleaned from the newly generated human genetic data are complemented by a detailed analysis of hepatitis B evolution. In addition to understanding the virus's past diversity and how that shaped present-day strains, the horizontal transfer of hepatitis B adds to our understanding of the prehistory of Europe by revealing contacts between groups which may not have left a detectable trace in the scant archaeogenetic record.

Manuscript A titled "Dynamic changes in genomic and social structures in 3rd millennium BCE central Europe" addresses the following questions:

- Were changes in archaeological culture accompanied by the arrival of non-locals?
- What was the central European genetic diversity present immediately prior to the arrival of "steppe" ancestry?
- When did "steppe" ancestry first arrive in central Europe, what was their genetic origin, and how did they interact with pre-existing local groups?
- What was the genetic origin of members of the Early Bronze Age Únětice culture?

Manuscript B titled "An Early Bronze Age community on the Amber Road – kinship and social behaviour in Mikulovice, Eastern Bohemia" addresses the following questions:

- What was the genetic origin of the Únětice people at Mikulovice?
- How was kinship organised?
- Is there a difference between how males and females relate to the cemetery?
- How does kinship relate to potential markers of wealth (i.e. grave goods)?

Manuscript C titled "Ten millennia of hepatitis B virus evolution" addresses the following questions:

- Where and when was the likely phylogeographic origin of hepatitis B and how did it spread around the world?

- Are population replacements in Europe correlated with the arrival of new hepatitis B strains?
- Is there evidence of exchange of hepatitis B strains across genetically differentiated populations?

Manuscript D titled “Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-Path Neolithic expansion to Western Europe” addresses the following questions:

- Can enriching for Y-chromosome targets improve sequencing efficiency and coverage on the Y-chromosome?
- Can new, phylogenetically informative variants be discovered?
- Can increased phylogeographic understanding of ancient Y-chromosomes elucidate past processes?

3. Overview of manuscripts and author's contribution

3.1 Manuscript A

"Dynamic changes in genomic and social structures in 3rd millennium BCE central Europe"

Luka Papac, Michal Ernée, Miroslav Dobeš, Michaela Langová, Adam B. Rohrlach, Franziska Aron, Gunnar U. Neumann, Maria A. Spyrou, Nadin Rohland, Petr Velemínský, Martin Kuna, Hana Brzobohatá, Brendan Culleton, David Daněček, Alžběta Danielisová, Miluše Dobisíková, Josef Hložek, Douglas J. Kennett, Jana Klementová, Michal Kostka, Petr Krištuf, Milan Kuchařík, Jana Kuljavceva Hlavová, Petr Limburský, Drahomíra Malyková, Lucia Mattiello, Monika Pecinovská, Katarína Petriščáková, Erika Průchová, Petra Stránská, Lubor Smejtek, Jaroslav Špaček, Radka Šumberová, Ondřej Švejcar, Martin Trefný, Miloš Vávra, Jan Kolář, Volker Heyd, Johannes Krause, Ron Pinhasi, David Reich, Stephan Schiffels, Wolfgang Haak.

Manuscript submitted to *Science Advances* on 24th of March 2021.
Manuscript accepted for publication in *Science Advances* on 20th of June 2021.

Manuscript A reports a densely sampled archaeogenetic transect from Neolithic, Eneolithic and Early Bronze Age Bohemia. By generating genetic data from 206 newly reported individuals and combining this with 65 previously published individuals, this study places Bohemia as one of the best studied regions in the world (from an archaeogenetic perspective). In generating these new data, we provide evidence for previously undetected genetic turnovers, including those associated with the appearance of the Funnelbeaker, Globular Amphora, and Únětice cultures in Bohemia. Genetic turnovers, both nuclear and Y-chromosomal, are detected also within archaeological cultures of the 3rd millennium BCE, including Corded Ware and Bell Beaker associated individuals. Importantly, insights into social structure and social processes are also obtained.

Author contributions:
W. Haak and M. Ernée conceived, designed and coordinated the study. M. Ernée, M. Dobeš, P. Velemínský, M. Kuna, H. Brzobohatá, D. Daněček, A. Danielisová, M. Dobisíková, J. Hložek, J. Klementová, M. Kostka, P. Krištuf, M. Kuchařík, J. Kuljavceva Hlavová, P. Limburský, D. Malyková, L. Mattiello, M. Pecinovská, K. Petriščáková, E. Průchová, P. Stránská, L. Smejtek, J. Špaček, R. Šumberová, O. Švejcar, M. Trefný, M. Vávra and R. Pinhasi provided archaeological material for study. W. Haak, **L. Papac**, M. Ernée, M. Dobeš and M. Langová collected samples. **L. Papac**, F. Aron, G. Neumann, M. Spyrou, N. Rohland performed lab work. **L. Papac**, B. Rohrlach, W. Haak, and S. Schiffels analysed the data. **L. Papac**, M. Ernée, M. Dobeš, W. Haak, J. Kolář and V. Heyd wrote the manuscript with input from all co-authors.

Overall, **L.Papac** contributed 70% to this manuscript.

3.2 Manuscript B

"An Early Bronze Age community on the Amber Road – kinship and social behaviour in Mikulovice, Eastern Bohemia."

Luka Papac, Michaela Langová, Ken Massy, Ronny Friedrich, Franziska Aron, Gunnar U. Neumann, Eliška Zazvonilová, Laura Arppe, Jan Cvrček, Sylva Drtikolová Kaupová, Vanessa Fairbank, Volker Heyd, Ladislava Horáčková, Petra Stránská, Ivo Světlík, Lenka Vargová, Petr Velemínský, Kateřina Vymazalová, Johannes Krause, Stephan Schiffels, Michal Ernée, Wolfgang Haak.

Manuscript in preparation for *Science Advances*.

Manuscript B reports a detailed investigation into an Early Bronze Age Únětice cemetery in Mikulovice, eastern Bohemia, Czech Republic. Mikulovice is situated on the Amber road, and ancient trade route linking the Baltic and Mediterranean seas and the cemetery is known for its rich and exotic grave goods. By combining archaeogenetic from 92 individuals with anthropological and archaeological data, we reconstruct biological kinship pedigrees and gain insights into aspects lifeways and behaviour in an earl Bronze Age community.

M. Ernée and W. Haak conceived, designed and coordinated the study. M. Ernée provided archaeological material for the study. W. Haak, **L. Papac**, M. Ernée, M. Langová and Eliška Zazvonilová collected samples. **L. Papac**, F. Aron and G. Neumann performed lab work. **L. Papac**, M. Ernée, M. Langová, K. Massy, R. Friedrich and W. Haak analysed the data.L. Arppe, J. Cvrček, S. Drtikolová Kaupová, V. Fairbank, V. Heyd, L. Horáčková, P. Stránská, I. Světlík, L. Vargová, P. Velemínský and K. Vymazalová provided archaeological and/or anthropological context. **L. Papac** and M. Ernée wrote the manuscript with input from all co-authors.

Overall, **L.Papac** contributed 70% to this manuscript.

3.3 Manuscript C

"Ten millennia of hepatitis B virus evolution"

Arthur Kocher, **Luka Papac**, …[see manuscript]…, Wolfgang Haak, Johannes Krause, Denise Kühnert.

Manuscript submitted to *Science* on 18th of March 2021.
Manuscript resubmitted to *Science* on 14th of June 2021 after minor revisions.

Manuscript C documents the origin, spread and evolution of hepatitis B virus across western Eurasia and the Americas. Reporting over 100 ancient hepatitis B samples spanning 10,000 years makes this the most detailed investigation of ancient hepatitis B virus to my knowledge. In addition to revealing insights into the virus' history, the horizontal transfer of hepatitis B virus also allows provides evidence for contacts between individuals of different cultural groups.

L.Papac conducted population genetic analyses, wrote part of Materials and Methods which outlines population genetic work done, and gave feedback on the manuscript.

Overall, **L.Papac** contributed 15% to this manuscript.

3.4 Manuscript D

"Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-Path Neolithic expansion to Western Europe"

Adam B. Rohrlach, **Luka Papac**, …[see manuscript]…, Alexander Herbig, Wolfgang Haak.

Manuscript submitted to *Scientific Reports* on 26th of February 2021.
Manuscript resubmitted to *Scientific Reports* on 18th of June 2021 after minor revisions.

Manuscript D reports a new capture method for enriching Y chromosomal DNA in ancient DNA libraries. The ability of the method to enrich for targeted Y chromosomal DNA is evaluated and the resulting higher resolution Y-chromosome data is used to resolve the phylogeny of the H2 Y haplogroup. In doing so, a phylogeographic pattern consistent with previous archaeological hypotheses about the routes by which farming spread to Europe is revealed, lending additional support to this hypothesis.

L.Papac helped in the computational analysis of Y chromosome diversity, conducted lab work, wrote part of Materials and Methods which outlines lab work done, and gave feedback on the manuscript.

Overall, **L.Papac** contributed 15% to this manuscript.

4. Manuscript A

Title

Dynamic changes in genomic and social structures in 3rd millennium BCE central Europe

One-sentence summary:

Archaeogenetic time transect in Europe unravels genetic and social changes before and after the arrival of "steppe" ancestry.

Authors

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Abstract
Europe's prehistory oversaw dynamic and complex interactions of diverse societies, hitherto unexplored at detailed regional scales. Studying 271 human genomes dated ~4900-1600 BCE from the European heartland, Bohemia, we reveal unprecedented genetic changes and social processes. Major migrations preceded the arrival of "steppe" ancestry and at ~2800 BCE three genetically and culturally differentiated groups co-existed. Corded Ware appeared by 2900 BCE, were initially genetically diverse, did not derive all "steppe" ancestry from known Yamnaya, and assimilated females of diverse backgrounds. Both Corded Ware and Bell Beaker groups underwent dynamic changes, involving sharp reductions and complete replacements of Y-chromosomal diversity at ~2600 and ~2400 BCE, respectively, the latter accompanied by increased Neolithic-like ancestry. The Bronze Age saw new social organization emerge amid a ≥40% population turnover.

Introduction

Archaeogenetics has revealed two major population turnovers in Europe within the last 10,000 years (*1–5*). The first, beginning in the 7th millennium before the common era (BCE), was associated with expanding Neolithic farming communities from Anatolia (*6*, *7*). European Early Neolithic farmers were initially genetically distinct from preceding hunter-gatherers (HG) and almost indistinguishable from Anatolian farmers (*8–10*), however incorporated HG ancestry into their gene pools over ensuing millennia (*3*, *11–13*).

The second major turnover occurred in the early 3rd millennium BCE with individuals of the Corded Ware (CW) culture (*3*, *4*, *8*). Of note, in what follows we use the co-occurrence of human skeletal remains and markers of archaeological cultures (e.g., grave goods, body orientation) to denote an association between individuals and an archaeological culture (e.g., "CW individuals"), although this may not reflect a unified social entity. The CW represents a significant cultural shift in central, northern, and northeastern Europe, bringing changes in economy, ideology, and mortuary practices (*14–22*). CW individuals were shown to be genetically distinct from culturally pre-CW people, having ~75% of their ancestry similar to Yamnaya individuals from the Pontic-Caspian steppe (*3*, *4*, *23–27*). This Yamnaya-like "steppe" ancestry then spread rapidly throughout Europe, reaching Britain, Ireland, the Iberian Peninsula, the Balearic Islands, Sardinia and Sicily before the end of the 3rd millennium BCE (*5*, *28–32*).

Despite the importance of the 3rd millennium BCE, our genetic understanding is mainly built upon studies with pan-European sampling strategies, with little emphasis on regional, high-resolution temporal transects (*3–5*, *8*). Consequently, many temporal and geographic sampling gaps remain, resulting in limited knowledge about the processes at the level of the societies and communities, how cultural groups interacted, influenced and gave rise to one another. Additionally, the use of small sample sizes to represent supra-regional archaeological phenomena, as well as the resulting oversimplified culture-historical interpretations, has drawn criticisms from archaeologists (*21*, *33–40*).

Unresolved questions concern the genetic and geographic origins of CW and Bell Beaker (BB) individuals, their relationship to one another and to Yamnaya individuals, as well as the origin of Early Bronze Age (EBA) Únětice individuals. Although it has been proposed that CW formed from a male-biased westward migration of genetically Yamnaya-like people (*23*, *41–44*), no overlap in Y-chromosomal lineages (with the exception of a few non-diagnostic I2) has been found between the predominantly R1a-carrying CW and mainly R1b-Z2103-carrying Yamnaya males. "Steppe" ancestry is also present in BB individuals (*5*), however, they predominantly carry R1b-P312, a Y-lineage not yet found among CW or Yamnaya males. Therefore, despite their sharing of "steppe" ancestry (*3*, *4*) and substantial chronological overlap (*45*), it is currently not possible to directly link Yamnaya, CW and BB groups as paternal genealogical sources for one another, particularly noteworthy in light of "steppe" ancestry's suggested male-driven spread (*23*, *41–43*) and the proposed patrilocal/patriarchal social kinship systems of these three societies (*46–48*).

Crucial to understanding the cultural, social and genetic transitions in 3rd millennium BCE Europe are densely settled regions which attest to the (co)existence of societies attributed to pre-CW (Baden, Globular Amphora), CW, BB and EBA (Únětice). Currently, no such region has been systematically studied from the archaeogenetic perspective. Situated in the heart of Europe and tightly nestled around the important Elbe river, the fertile lowlands of Bohemia, the western part of today's Czech Republic, witnessed many major supra-regional archaeological phenomena (Table S1, Fig. 1, Fig. S1, Supplementary

Information). Dense agrarian settlement of Bohemia began after ~5,400 BCE (*49*, *50*) with the arrival of early Neolithic farmers (Linearbandkeramik-LBK, later Stichbandkeramik-STK and Lengyel). They were succeeded by manifold societies of the Eneolithic (~4400-2200 BCE), associated with more than a dozen archaeological cultural groups including Jordanów, Michelsberg, Funnelbeaker, Baden, Řivnáč, Globular Amphora (GAC), Early and Late CW, and BB (Table S1) (*50*). The Eneolithic witnessed significant innovations (metallurgy, the wheel, wagon and plough, fortified hillforts, burial mounds) (*51*–*53*) and was succeeded by the globalized EBA Únětice culture, geographically centered around Bohemia.

In addition to material and technological developments, ideological changes, as manifested through mortuary behavior, are also evident (*54*). Although relatively common during the Funnelbeaker period (~3800–3400 BCE, n=~100 known graves in Bohemia) (*55*), regular graves almost disappear from the succeeding Baden, Řivnáč and GAC periods (Middle Eneolithic, ~3500-2800 BCE, n=~20) (*56*). Single graves, but now with strict gender differentiation in body position and grave goods, reappeared in abundance with CW from ~2900 BCE (n=~1500) (*50*, *57*) and continued with BB (n=~600) from ~2,500 BCE (*58*), who developed and maintained important differences from the preceding CW. The EBA Únětice culture (*59*, *60*) continued with single graves (n=~4000-5000), but now again without gender differentiation in body position.

In order to better understand these transitions, we analyzed a high-resolution archaeogenetic time transect of 271 (206 newly reported and 65 previously published) individuals (Fig. 1, Fig. S1, Table S2-S4, Supplementary Materials) from the northern part of Bohemia. Through dense genetic sampling from geographically and temporally overlapping archaeological cultures, we aim to i) address whether cultural changes in the Eneolithic and EBA of central Europe were driven by an influx of non-locals, ii) characterize the central European genetic diversity immediately prior the appearance of CW, iii) date when individuals with Yamnaya-like “steppe” ancestry first appeared in central Europe, and understand their genetic origin and social structure iv) characterize the nature and extent of biological exchange between the “locals” and “migrants” after appearance of CW, and v) identify social transformations linked to genetic and archaeological changes.

Results

General sample overview

We screened 261 prehistoric individuals (Table S3) from 37 sites (Table S2) for ancient human DNA preservation, of which 219 individuals were enriched for 1,233,013 ancestry informative sites in the human genome(“1240K capture panel”) (*8*). After enrichment, individuals with fewer than 30,000 sites covered (on 1240K) or signs of contamination were removed (n=13), resulting in a dataset of 206 newly reported individuals. We combined our dataset with 65 previously published individuals (*5*, *61*, *62*) from Bohemia (with >30,000 covered sites on 1240K, Table S4) and wider (Table S5), thereby extending the total number of published Bohemian Neolithic and pre-CW Eneolithic individuals from 7 to 58 (Fig. S2), CW individuals from 7 to 54 (Fig. S3), BB individuals from 40 to 64 (Fig. S4) and EBA individuals from 11 to 95 (Fig. S5). Crucially, we substantially expand the sample size of individuals around the time of CW formation (~3200-2600 BCE, from n=1 to n=50, Fig 1B), i.e. the last pre-CW (Baden, Řivnáč, GAC, from n=0 to n=18) and the early CW (from n=1 to n=32) individuals, allowing us to directly study the origin of CW in central Europe, the nature of their migration, and social interactions with co-existing pre-CW people. First degree relatives were excluded

from allele frequency-based analyses (*f*-statistics, *qpWave*, *qpAdm*, DATES, and Y-chromosome analyses; Table S4, see methods section). We also report 140 new radiocarbon dates to aid in finer temporal resolution, allowing us for the first time to study the genetic changes between early and late phases of important 3rd millennium BCE cultural groups (e.g., CW, BB, Únětice, Table S4, Table S6).

Bohemia before Corded Ware (pre-CW, before ~2800 BCE)

We first assessed the genome-wide data by projecting the ancient individuals from Bohemia onto the first two axes of a principal components analysis (PCA) constructed from 1,141 modern-day West Eurasian individuals (Table S7). In the resulting PCA plot (Fig. 2A), all (n=58) pre-CW individuals from Bohemia plot between Anatolia_Neolithic and Western Hunter-Gatherers (WHG), in close proximity to published culturally pre-CW individuals from central Europe (*3*, *8*, *11*, *13*, *25*). This suggests an absence of "steppe" ancestry, which we formally confirmed using *qpAdm* modelling (Table S8), revealing that pre-CW individuals from Bohemia can be largely modelled as two-way mixtures of Anatolia_Neolithic and WHG (Fig. 3A, Table S8-S9, Fig. S6). The percentage of HG ancestry is positively correlated with time (Spearman's rank correlation r=0.39, p<0.004), showing that the previously reported trend of increasing HG ancestry during the Neolithic also took place in Bohemia (*3*, *11*). We found this HG ancestry increase to be best modelled as a two-stage linear process (Fig. 3A, Table S8, Supplementary Methods), with an increase in HG ancestry during the 5th millennium BCE, followed by stasis (non-significant slope) thereafter (Fig. 3A, Supplementary Methods).

In order to gain insight into the process(es) by which HG ancestry was incorporated into the gene pool of pre-CW individuals from Bohemia, we used *qpAdm* to model each pre-CW cultural group as a three-way mixture of Anatolia_Neolithic, Loschbour and Körös_HG as well as *DATES* to estimate the introgression date of incorporated HG ancestry (Fig. 3B, Table S10-S11).

Under a scenario of population continuity with sequential incorporation of HG ancestry, the mean date of introgression, as indicated by the grey intervals in Fig. 3B (right), for succeeding cultures is expected to become more recent through time. Conversely, under population continuity without incorporation of further HG ancestry, the admixture date should be similar for successive cultural groups who have similar HG proportions.

Our results indicate two cultural transitions for which either of these expectations is not met. First, although Bohemian Jordanów and Funnelbeaker have similar amounts of WHG ancestry [f4(Mbuti.DG, WHG; Jordanów, Funnelbeaker) ~0, Z-score 0.96], the estimated date of WHG introgression for Funnelbeaker is significantly earlier (5,079-4,748 BCE) than for Jordanów (4,636-4,310 BCE) (Fig. 3B, Table S11), consistent with Bohemian Funnelbeaker individuals being derived from a different population (whose HG ancestry was incorporated further back in time) which superseded the Jordanów population in Bohemia. This transition between Jordanów and Funnelbeaker is corroborated by three additional observations. First, an f4-statistic of the form f4(Mbuti.DG, Bohemia-Funnelbeaker; Bohemia-Jordanów, Germany-Funnelbeaker) is positive (Z-score 3.14), revealing significantly greater genetic affinity of Bohemian Funnelbeaker to Funnelbeaker individuals from Saxony-Anhalt than to the preceding local Jordanów individuals. Conversely, f4(Mbuti.DG, Bohemia-Jordanów; Germany-Funnelbeaker, Bohemia-Funnelbeaker) is consistent with 0 (Z-score 1.03), suggesting phylogenetic cladality between Bohemian and German Funnelbeaker with respect to Bohemia-Jordanów individuals. Second, Bohemia-Jordanów individuals can be

modelled as a two-way mixture of Anatolia_Neolithic and Körös_HG but not Anatolia_Neolithic and Loschbour, while the opposite is true for Bohemia-Funnelbeaker (Table S12). This suggests different affinities, in addition to the different introgression dates, of the HG ancestries in Bohemian Jordanów and Funnelbeaker cultural groups. Third, *qpWave* does not support cladality between Bohemian Jordanów and Funnelbeaker (p=0.00679), while cladality between Bohemian and German Funnelbeaker cannot be rejected (p=0.88, Table S13). Taken together, these results indicate a largely (significantly more than 50%) non-local genetic origin of Bohemian Funnelbeaker individuals.

The second such case can be seen in the Řivnáč to GAC cultural transition. GAC individuals carry the most HG ancestry among pre-CW cultural groups from Bohemia (25.7% ±1.4), significantly more than Řivnáč individuals [f4(Mbuti.DG, WHG; Řivnáč, GAC) >> 0, Z-score 4.46]. However, the estimated date of HG admixture in GAC is not later than in Řivnáč individuals (Fig. 3B, Table S11), suggesting GAC individuals do not descend from a recent mixture of Řivnáč and a HG source, but instead constituted a recent, non-local incursion in Bohemia from a region which received more HG gene-flow (e.g., Poland (*13*, *63*)), in accordance with interpretations of archaeological evidence (*56*).

A distinct genetic origin for Řivnáč and GAC individuals is further supported by PCA and *qpAdm* modelling. From PCA we find that, with the exception of TUC003, Řivnáč and GAC individuals form distinct clouds (Fig. 3C). This is confirmed by *qpAdm* modelling where GAC individuals can be modelled as a mixture of Anatolia_Neolithic and Loschbour but not Anatolia_Neolithic and Körös_HG, while the opposite is true for Řivnáč individuals (Table S14). Consequently, Řivnáč and GAC individuals are distinguishable based on the amount and source of HG ancestry, suggesting that Bohemia was inhabited by genetically differentiated groups of Řivnáč and GAC individuals at the time of CW appearance. The Řivnáč outlier (TUC003) also raises the interesting possibility of an individual born into a GAC but buried in a Řivnáč cultural context.

Importantly, among the 16 Řivnáč and GAC individuals who are contemporaneous with or post-date the appearance of CW in Bohemia (Fig. 1B), we find no detectable traces of “steppe” ancestry (Fig. 2A, Table S8), suggesting that biological exchange from CW/Yamnaya into culturally pre-CW people (e.g., Řivnáč, GAC) was low, possibly non-existent. “Steppe” ancestry co-appears with CW individuals in early 3rd millennium BCE Bohemia.

Corded Ware

We report genomic data from the earliest CW individuals to date, including STD003 (northwestern Bohemia, 3010-2889 BCE), VLI076 (central Bohemia, 3018-2901 BCE), OBR003 (central Bohemia, 2911-2875 BCE) and PNL001 (eastern Bohemia, 2914-2879 BCE), showing that CW was widespread across Bohemia by 2900 BCE. The early radiocarbon dates are also supported by these individuals’ genetic profiles, who occupy the most extreme positions on PC2 (Fig. 2B), as expected under a scenario of the earliest CW being migrants from the east who mixed with locals, resulting in intermediate PC2 positions in later generations.

To explore the formation of the Bohemian CW gene pool, we grouped CW individuals with “steppe” ancestry and mean age >2600 BCE (n=27) into a Bohemia_CW_Early group, and the rest (n=21) into Bohemia_CW_Late (Table S4). We found poor statistical support (p<0.005) for modelling Bohemia_CW_Early as a two-way mixture of any known Yamnaya source and any local Bohemian or non-local pre-CW source from

Poland, Ukraine, Hungary, or Germany (Table S15). When using distal sources as proxies for the Neolithic ancestry (Anatolia_Neolithic and a range of HG sources) we found no strong support ($p<0.05$) for all but one of the three-way distal models (Table S16). However, this one statistically supported model results in a previously unobserved ratio of Neolithic ancestry in Europe (i.e. a Neolithic population of ~1:1 ratio of Anatolia_Neolithic:Sweden_Motala_HG). In addition, when modelling early CW individually as 'standard' three-way mixtures of Anatolia_Neolithic, WHG and Yamnaya_Samara (*3*), we find in 37% (10/27) of cases the model lacks strong support ($p<0.05$ or infeasible, Fig. S6, Table S9).

In order to explore why two-way proximal models between any Yamnaya and a European Neolithic source are insufficient in explaining Bohemia_CW_Early genetic diversity, we tried adding a third source to obtain better model fits. We find that when either one of Latvia_MN, Ukraine_Neolithic or PittedWare is added as a source, almost all (280/285) model fits (p-values) improve, and most of them by several orders of magnitude (Table S17). While all (n=95) two-way proximal models lack strong support ($p<0.05$, Table S17), the addition of either Latvia_MN (57/95 supported models), Ukraine_Neolithic (53/95 supported models) or PittedWare (32/95 supported models) to the sources drastically increases the number of supported models (Table S17). These results show the presence of excess Latvia_MN/Ukraine_Neolithic/PittedWare-like ancestry in Bohemia_CW_Early relative to all known Yamnaya and central European Neolithic groups. Our models suggest this ancestry accounts for ~5-15% of the Bohemia_CW_Early gene pool (Table S17). Increases in model fits with either of these third sources are also observed when modelling Bohemia_CW_Late and Germany_Corded_Ware, suggesting this ancestry to be present also in later central European CW (Table S18-19), and is consistent with allele sharing f4-statistics, which show that CW groups share more alleles with ancient northeast European groups than do Yamnaya (Table S20-21).

We provide the first genomic data from CW individuals without "steppe" ancestry, thereby elucidating the social processes of interaction between CW and pre-CW people. Observing only females (4/4) among early CW individuals without "steppe" ancestry (Fig. 2B, Fig. 3C) suggests the process of assimilating pre-CW people into early CW society was female-biased. Two of these females (STD003, VLI008) plot in close PCA space to GAC individuals from Bohemia and Poland (Fig. 3C). When grouped together, we find that STD003+VLI008 share more genetic affinity with Bohemian GAC than with Bohemian Řivnáč [f4(Mbuti.DG, STD003+VLI008; Bohemia-GAC, Bohemia-Řivnáč) < 0, Z-score -2.32]. Interestingly, these two females are not genetically closer to Bohemian compared to Polish GAC individuals [f4(Mbuti.DG, STD003+VLI008; Bohemia-GAC, Poland-GAC) ~ 0, Z = 0.5], meaning that a non-local, (north)eastern origin (e.g., Poland) cannot be ruled out. In addition, VLI009 and VLI079 fall outside of the sampled Bohemian Middle Eneolithic (Baden, Řivnáč and GAC) genetic variation in PCA, carrying significantly more HG ancestry (Fig. 3C; Table S22), suggesting that a large proportion (50%, or higher when including STD003/VLI008) of the genetically pre-CW females of the early CW society originated from outside Bohemia.

We find Bohemia_CW_Late carries significantly more pre-CW-Eneolithic-like ancestry compared to Bohemia_CW_Early (Table S23), however this signal is lost when early CW females without "steppe" ancestry are included (Table S24). This additional pre-CW-Eneolithic-like ancestry in Bohemia_CW_Late (relative to Bohemia_CW_Early) is poorly modelled as coming from local sources (Table S25), suggesting non-local genetic influences

on the Bohemian CW gene pool through time. This is consistent with the genetically pre-CW females originating from outside of Bohemia, and is supported by the finding that Bohemia_CW_Early (including females without "steppe" ancestry) and Bohemia_CW_Late are not cladal in *qpWave* analysis (Table S26), despite having similar amounts of pre-CW-Eneolithic-like ancestry.

In addition to autosomal genetic changes through time, we observe a sharp reduction in Y-chromosomal diversity going from five different lineages in early CW to a dominant (single) lineage in late CW (Fig. 4A). We used forward simulations to explore the demographic scenarios which could account for the observed reduction in Y-chromosomal diversity. Performing one million simulations of a population with a starting frequency of R1a-M417(xZ645) centered around the observed starting frequency in Bohemia_CW_Early (3/11, 0.27), we assessed the plausibility of this lineage reaching the observed frequency in Bohemia_CW_Late (10/11, 0.91) in the time frame of 500 years under a model of a closed population and random mating (Materials & Methods). We reject the "neutral" hypothesis, i.e. that this change in frequency occurred by chance, given a wide range of plausible population sizes. Instead, our results suggest that R1a-M417(xZ645) was subject to a non-random increase in frequency, resulting in these males having 15.79% (4.12%-44.42%) more surviving offspring per generation relative to males of other Y-haplogroups. We also find this change in Y-chromosome frequency is extreme compared to the changes in allele frequencies at fully covered autosomal 1240K sites ($p<0.0003$) within the same males, suggesting a process which disproportionately affected Y-chromosomal compared to autosomal genetic diversity, ruling out a population bottleneck as the likely cause. Our results suggest that the Y-lineage diversity in early CW males was supplanted by a non-random process (selection, social structure, or influx of non-local R1a-M417[xZ645] lineages) that drove the collapse in Y-chromosomal diversity. A simultaneous decline of Y-chromosomal diversity dating to the Neolithic has been observed across most extant Y-haplogroups (*64*), possibly due to increased conflict between male-mediated patrilines (*65*). We view that changes in social structure (e.g., an isolated mating network with strictly exclusive social norms) could be an alternative cause but would be difficult to distinguish in the underlying model parameters.

The greatest genetic differentiation within early CW individuals can be found at Vliněves. The f_{st} between the three highest and three lowest early CW individuals on PC2 from Vliněves is greater than pairwise comparisons of all modern-day European populations (Fig. 5, Table S27).

Bell Beaker

The earliest BB individuals occupy a similar position in PCA as CW individuals (Fig. 4B, Fig. S7), suggesting a degree of genetic continuity. To explore the genetic origin of early BB individuals (Bohemia_BB_Early, mean date >2400 BCE, n=3), we modelled them as a two-way mixture between preceding and contemporaneous cultural groups. We found support for a local origin, although non-local alternatives cannot be ruled out (Table S28). However, our Bohemia_BB_Early group consists of only three (female) individuals, and is therefore likely limited in representativeness and resolution to discern source populations.

We find that late BB individuals (Bohemia_BB_Late, mean date ≤2400 BCE, n=56) carry significantly more Middle Eneolithic-like ancestry compared to Bohemia_BB_Early (Table S29). To explore this genetic shift, we modelled the ancestry of Bohemia_BB_Late as a two-way mixture of Bohemia_BB_Early and local Middle Eneolithic sources (Table S30),

finding support for an additional ~20% local Middle Eneolithic-like ancestry in late compared to early BB.

We observe a closer phylogenetic relationship between the Y-chromosome lineages found in early CW and BB, than in either late CW or Yamnaya and BB. R1b-L151 is the most common Y-lineage among early CW males (6/11, 55%), and one branch ancestral to R1b-P312 (Fig. 4A), the dominant Y-lineage in BB (*5*). Although it is not possible to determine whether the P312 mutation(s) occurred in one of the early CW R1b-L151 males from Bohemia, we note that most Bohemian BB males are further derived at R1b-L2/S116 (R1b1a1a2b1), in contrast to BB males from England, several of whom are derived at R1b-L21(R1b1a1a2c1), showing that English and Bohemian BB males cannot be descendants of one another, but rather diversified in parallel. A scenario of R1b-P312 originating somewhere between Bohemia and England, possibly in the vicinity of the Rhine (*66*, *67*), followed by an expansion northwest and east is compatible with our current understanding of the phylogeography of ancient R1b-L151-derived lineages.

Early Bronze Age - Únětice Culture

The transition to the EBA in Bohemia is associated with a positive shift in the coordinates of PC2, relative to preceding late BBs (Fig. 4B, Fig. S7, Table S31). Admixture f3 statistics are most negative when EHG (Eastern hunter-gatherer) or WSHG (West Siberian hunter-gatherer) are used as a second source in addition to the geographically and temporally proximal Bohemia_BB_Late (Table S32), suggesting a northeastern contribution to Bohemia_Únětice_preClassical. To find a suitable proxy for a potential additional source population, we modelled Bohemia_Únětice_preClassical as a two-way mixture of local Bohemia_BB_Late and various sources more positive on PC2 (Table S33). We reject mixture models involving Bohemia_BB_Late and Yamnaya (Samara p=5.3e-10, Kalmykia p=5.8e-10, Ukraine p=7.3e-12, Caucasus 3.2e-15) or Bohemia_BB_Late and CW (early p=1.1e-4, late p=5.4e-6). We fail to reject a two-way mixture model of 63.5% Bohemia_BB_Early and 36.5% Bohemia_BB_Late (p=0.29), suggesting a large (63.5%) contribution from an early BB lineage which was largely unsampled during the late BB phase (2400-2200 BCE), but represents a potential new lineage at the dawn of the Bronze Age. The Y-chromosomal data suggests an even larger turnover. A decrease of Y-lineage R1b-P312 from 100% (in late BB) to 20% (in pre-classical Únětice) implies a minimum 80% influx of new Y-lineages at the onset of the EBA.

However, aware of the limited resolution of Bohemia_BB_Early (small sample size, low resolution, large standard errors), we explored alternative models for pre-classical Únětice individuals. All model fits improve when Latvia_BA is included in the sources, resulting in two additional supported models (Table S33). A three-way mixture of Bohemia_BB_Late, Bohemia_CW_Early and Latvia_BA (p-value 0.086) supports a more conservative estimate of 47.7% population replacement and, importantly, also accounts for the Y-chromosomal diversity found in pre-classical Únětice, with R1b-P312 from Bohemia_BB_Late, R1b-U106 and I2 from Bohemia_CW_Early, and R1a-Z645 from Latvia_BA (Fig. 4A).

Although the geographic origin of this new ancestry cannot be precisely located, three observations offer clues. First, the Latvia_BA ancestry that improves all model fits (Table S33) suggests an ultimate northeastern origin. Second, Y-haplogroup R1a-Z645 appears in Bohemia (and wider central Europe) for the first time at the beginning of the EBA, a lineage previously fixed in Baltic and common in Scandinavian CW males (*23*, *24*),

supporting a north/northeastern genetic contribution. Third, an Únětice genetic outlier (VLI051, male, Y-haplogroup R1a-Z645, Table S34) resembles individuals from Bronze Age Latvia (Fig. 2D) (*68*), providing direct evidence for migrants from the northeast.

We also detect a genetic shift in the transition from pre-classical to classical Únětice, reflected in the decrease in PC2 coordinates for Únětice individuals dated after ~2000 BCE (Fig. 4B, Fig. S7) and confirmed using *qpWave* (Table S35) and f4 statistics (Table S36). Bohemia_Únětice_Classical can be modelled as a mixture of Bohemia_Únětice_preClassical and a local Eneolithic source (Table S37). In contrast to the genetic shift between late BB and pre-classical Únětice, the Y-lineage diversity remains similar throughout both Únětice phases, suggesting assimilation and subtler social changes.

Discussion

The high-resolution genetic time transect in Bohemia, allowing for the first time early and late phases of cultural groups to be divided and studied separately (e.g., CW, BB, Únětice), elucidates several major processes before and after the arrival of "steppe" ancestry (Fig. 6). Our dense sampling allows detection of novel, important and perhaps 'unexpected' changes within cultural groups (e.g., CW, BB), if they are seen through a strict cultural-historical lens. Previous studies have largely been interpreted as revealing major migrations at the beginning and end of the Neolithic (i.e. periods where the incoming groups were genetically very distinct), however our results reveal additional large genetic turnovers. By sampling consecutive and partially contemporaneous cultural groups, we show for the first time that the spread of Funnelbeaker and GAC (*69*, *70*), as well as the origin of Únětice, involved large genetic shifts over short time periods, likely explained by migrations.

We show that early CW were genetically exceptionally diverse, some resembling GAC and Yamnaya, with a few also falling outside of previously sampled central European Neolithic genetic diversity. Such a strikingly diverse signal is likely the result of the agglomeration of people from diverse cultural and linguistic backgrounds into an archaeologically similar, but polyethnic or plural society. Important factors in ethnic identity include ancestry, history, ideology, and language (*71*, *72*). The level of genetic differentiation (i.e. time since common ancestor) between early CW individuals with high and no "steppe" ancestry implies long biological isolation and hence different histories. The finding of GAC-like and Yamnaya-like genetic profiles in early CW suggests integration of people who came from ideologically diverse socieities (i.e. neither GAC nor Yamnaya practiced strong gender differentiation in mortuary practices, unlike CW). It is likely that GAC and CW/Yamnaya individuals spoke different languages (*3*, *4*, *43*), meaning that early CW society in Bohemia encompassed people who had demonstrably different histories, likely originating from ideologically diverse cultures, who spoke different mother tongues.

The assimilation process of individuals without "steppe" ancestry into early CW society was female-biased (*43*). However, finding females also among individuals with highest amounts of "steppe" ancestry (3/5, Fig. 2B) suggests they were also well represented among migrating CW individuals (in contrast to (*43*)), or perhaps assimilated from nearby Yamnaya groups (e.g., Hungary). Finding individuals without "steppe" ancestry in early CW contexts (n=4) is more common than individuals with "steppe" ancestry in pre-CW contexts (e.g., GAC, n=0). This pattern of asymmetric gene flow between the contemporaneous GAC and CW may reflect newcomers (CW groups) having more benefit from incorporating people with important local knowledge (i.e. from pre-CW cultural

contexts) into their communities. The archaeological record shows continuity of such knowledge (e.g., pottery production, lithic raw materials) in several regions (*22*, *67*, *73*).

Vliněves is crucial for elucidating interactions between individuals with high and no "steppe" ancestry. This site yields the earliest dated CW (VLI076, 3018-2901 BCE) who is also genetically most differentiated from pre-CW individuals, while 20% (3/15) of the sampled early CW from Vliněves had no "steppe" ancestry. Intriguingly, we observe no archaeological differences between CW graves of individuals with and without "steppe" ancestry from two sites (Vliněves and Stadice, see Supplementary Information), suggesting full integration of genetically, and likely ethnically, diverse individuals within the same archaeological culture.

Finding Latvia_MN-like ancestry in early CW, in conjunction with the absence of Y-chromosomal sharing between early CW and Yamnaya males, suggests a limited or indirect role of known Yamnaya in the origin and spread of CW to central Europe. Our results allude to either a northeast European Eneolithic forest steppe contribution to early CW (a region consistent with some interpretations of the archaeological evidence (*57*)) or a hitherto unsampled steppe population who carried excess Latvia_MN-like ancestry, a scenario less likely given the high degree of genetic homogeneity among 3000 BCE steppe groups (e.g., Yamnaya and Afanasievo separated by ~2500 km but genetically almost indistinguishable (*4*, *61*)). As much of 4000-2500 BCE (north)eastern Europe remains unsampled, inferring the precise geographic origin of early CW individuals remains elusive.

Since social kinship systems influence patterns of genetic diversity (*13*, *42*, *48*, *74*), it is likely that several different kin systems existed in 3rd millennium BCE central Europe. The highly diverse genetic profiles (both nuclear and Y-chromosomal) of early CW suggests a different social organization to late CW and BB, whose Y-chromosome pattern is indicative of strict patrilineality. This suggests that different cultural groups, in addition to using various forms of material culture and mortuary practices, likely also conformed to different ideologies as expressed in their mating pattern and/or social organisation. This is supported by the finding of completely non-overlapping Y-chromosome variation between the partially contemporaneous late CW and BB, indicating a large degree of paternal mating isolation between these two groups, even when found at the same site (e.g., Vliněves).

The onset of the pre-classical Únětice was accompanied by a ≥40% nuclear and ≥80% Y-chromosomal contribution ultimately originating from the northeast and breaking down the gender-differentiated mortuary practices and strict patrilineality of late CW and BB. This was neither evident in the burial customs nor in the material culture, but could represent the underlying connection to the Baltics, the ultimate source of EBA amber in Bohemia associated with the later emerging Amber Road (*75*–*77*). Therefore, our results suggest two main periods (early CW and early Únětice) of genetic influence from the northeast, much of which remains unsampled in the European archaeogenetic record (e.g., Belarus).

Our results reveal a complex and highly dynamic history of Neolithic to EBA central Europe, during which migration and the movement of people facilitated abrupt genetic and social changes. Large-scale demic expansions occurred multiple times before and after the appearance of "steppe" ancestry in Europe. Early CW society was diverse and emerged amid a strong cultural and genetic transition, involving males and females of diverse origins and likely ethnicities. Genetic shifts occurred within CW, BB and EBA societies despite continuity in material culture. Cultural affiliations played a major role in 3rd millennium BCE social behaviors, which ultimately changed with the influx of new people over time.

Although the impact of social processes is observable in patterns of genetic diversity,

further interdisciplinary research is required to characterize the drivers of these changes, both at a micro- and macro-regional level.

Materials and Methods

Processing sites for the newly reported individuals

Most (186/206, 90.2%) of the newly reported individuals were entirely processed at the Max Planck Institute for the Science of Human History in Jena, Germany, the full details of sampling and ancient DNA wet laboratory work and bioinformatic processing are summarized in what follows. The individuals from the site Makotřasy were initially sampled and processed into powder at the University of Vienna, followed by subsequent lab work, bioinformatic and ancient DNA analysis at Harvard Medical School following previously described protocols (*61*).

Sampling

In total, 389 pars petrosa, teeth and bones from 261 individuals were processed as part of this study. Upon introduction into the clean room facilities at the Max Planck Institute for the Science of Human History in Jena, Germany, all samples were wiped with 5% bleach and UV irradiated for 20 minutes on each side. Teeth were sampled by removing the crown followed by drilling into the pulp chamber to create bone powder. Pars petrosa were sampled by drilling into their dense region (*78*) to create bone powder. Between 50-100mg of resulting bone powder from each sample was collected in different 2mL Biopure tubes (one tube per sample) and used in subsequent DNA extraction.

DNA Extraction

One mL of extraction buffer (containing 0.9mL 0.5M EDTA, 0.025mL 0.25mg/mL Proteinase K and 0.075mL UV HPLC-water) was added to Biopure tubes containing bone powder. Biopure tubes were then sealed with Parafilm and incubated overnight on a rotating wheel at 37°C. After incubation, Biopure tubes were spun for two minutes at 18500 relative centrifugal force (rcf), separating the soluble from insoluble parts of the resulting solution. The soluble part was transferred to a 50mL falcon tube containing 10mL binding buffer and 400µL sodium acetate (3M, pH 5.2). Resulting mixture was transferred to a High Pure Extender Assembly (HPEA) falcon tube which was centrifuged at 1500 revolutions per minute in a 50 mL Thermo Scientific TX-400 Swinging Bucket Rotor for 8 minutes. The column from each HPEA tube was removed and inserted into a fresh collection tube and centrifuged at 18500 rcf for 2 minutes. Four hundred and fifty µL of wash buffer from the high pure viral nucleic acid kit (HPVNAK) was added to each column which was then centrifuged at 8000 rcf for 1 minute. Columns were then removed and placed into new collection tubes. Another round of washing was performed whereby 450µL of wash buffer from the HPVNAK was added to each column and centrifuged at 8000 rcf for one minute. Columns containing washed DNA were then transferred to 1.5mL siliconised tubes. Fifty µL of TET was added to the centre of columns, the columns were then incubated at room temperature for 3 minutes and centrifuged at 18500 rcf for 1 minute. Another 50µL of TET was added to the centre of columns, after which they were centrifuged once more at 18500 rcf for 1 minute. The resulting 100µL DNA extracts were stored at -20°C until further processing.

DNA Libraries and In-solution Capture

Twenty-five µL of DNA extract was used for the construction of (in most cases) double-stranded UDG-half treated DNA libraries. UDG repair was performed by adding DNA extract

to a 25µL mastermix containing 6µL 10x Buffer Tango, 6µL 10mM ATP, 0.5µL 20mg/ml BSA, 0.2µL 25mM each dNTPs, 3.6µL 1U USER enzyme and 8.7µL UV HPLC-water. Resulting mixture was incubated at 37°C for 30 minutes followed by 12°C for one minute. Inhibition of UDG treatment was achieved through the addition of 3.6µL 2U UGI to each tube followed by incubation at 37°C for 30 minutes and again at 12°C for one minute. Blunt-end repair was performed by adding 3µL 10U T4 Polynucleotide Kinase and 1.65µL 3U T4 DNA Polymerase and incubating the resulting mixture at 25°C for 20 minutes, then at 12°C for 10 minutes. Blunt-end repaired mixture was purified using MinElute kit and eluted in 20µL Elution buffer (EB) containing 0.05% tween. Illumina adapters were ligated onto DNA molecules through the mixture of 18µL eluate from the previous step with 20µL of 2x Quick Ligase Buffer, 1µL 10µM Adapter Mix and 1µL 5U Quick Ligase. Resulting mixture was incubated at 22°C for 20 minutes followed by purification with MinElute kit and elution in 22µL EB containing 0.05% tween. Adapter fill in reaction was performed by adding 20µL of eluate from previous step to 4µL 10x Isothermal buffer, 0.2µL 25mM each dNTPs, 2µL 8U Bst 2.0 Polymerase, and 13.8µL UV HPLC-water followed by incubation at 37°C for 30 minutes and 80°C for 10 minutes. Resulting libraries were stored at -20°C until further processing. Unique library-specific indexes were added to the 5' and 3' ends of molecules in each library through an indexing PCR reaction. Each library was split into four separate indexing PCR reactions which were carried out using 10µL 10x Pfu Turbo Buffer, 1.5µL 20mg/ml BSA, 1µL 25mM each dNTPs, 1µL 2.5U Pfu Turbo Polymerase, 73.5µL UV HPLC-water, 2µL 10µM P5 index, 2µL 10µM P7 index and 9µL of DNA library. Amplification was achieved through an initial denaturation at 95°C for 2 minutes, followed by 10 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute, followed by 72°C for 10 minutes. Resulting indexed libraries of the same sample were pooled and purified using MinElute purification kit. Purified libraries were quantified using qPCR and amplified to 10^13 copies. Amplified libraries were shallow shotgun sequenced (~5 million reads) on an Illumina Hiseq or Nextseq platform to estimate the general human DNA content, presence of ancient DNA damage and mitochondrial:nuclear coverage ratio. Libraries with >0.1% endogenous human DNA and >5% C-to-T misincorporations at the 5' end were chosen for 1240K capture (*8*) and mitochondrial DNA (mtDNA) capture. In cases where more than one library from the same individual satisfied criteria for capture, the better quality (higher endogenous DNA content) library was used for 1240K and mtDNA capture.

Sequencing

Post-capture libraries were single-end (75 cycles) or paired-end sequenced (2x50 or 2x75) on HiSeq or NextSeq Illumina platforms to a depth of 20-50 million reads per library. Resulting sequence data was processed through EAGER (v1.92.38) (*79*). Illumina adapters were removed using AdapterRemoval (v2.2.0) (*80*) and in case of paired-end sequencing, corresponding reads from the same template molecule with minimum 11 base pairs of overlap were merged. Fastq files of merged and unmerged reads were concatenated and reads shorter than 30 base pairs were discarded. Processed reads were mapped to the human reference genome (hg19) using BWA-aln and BWA-samse (v0.7.12) (*81*) applying maxdiff (-n) 0.01 and seeding turned off (-l 10000). Resulting bam files were sorted and duplicate reads were removed using DeDup (v0.12.1) (https://github.com/apeltzer/DeDup). Damageprofiler (v0.3.10) (https://github.com/Integrative-Transcriptomics/DamageProfiler) was used to calculate rates of misincorporation in read termini of DNA fragments in our

captured libraries. BAM files had the last 3 bases from both 5' and 3' ends of reads and corresponding base quality scores masked for downstream analyses (*82*).

Sex determination and authentication

The genetic sex of each sample (bam file) was determined by calculating the normalized mean coverage on the X (mean X coverage / mean autosome coverage) and Y (mean Y coverage / mean autosomal coverage) chromosomes (*83*). Samples with normalized mean Y coverage values greater than 0.2 were assigned male. Contamination was estimated in males by calculating the rate of heterozygosity on their X chromosome (*84*). In addition, we used schmutzi to estimate the mitochondrial contamination in all libraries (*85*). Schmutzi was run on BAM files resulting from mapping 1240K capture sequencing data to the human mitochondrial reference genome. In cases where 1240K data was not enough to give an mtDNA contamination, we ran schmutzi on the mtDNA capture data mapped to the human mitochondrial reference genome.

Genotyping

We used samtools (v1.3) (*86*) mpileup and pileupCaller from the sequenceTools (v1.4.0.2) package (https://github.com/stschiff/sequenceTools) to call pseudo diploid genotypes by sampling a random high-quality allele (base quality ≥ 30 and mapping quality ≥ 30) from each of the 1240K sites (*8*). Newly generated genotype data for this study was then merged to a compiled dataset of previously published ancient and modern worldwide populations (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data) (v42.2) using mergeit (v2450) from the EIGENSOFT package (https://github.com/DReichLab/EIG).

Mitochondrial and Y chromosome haplogroups

Mitochondrial haplogroups were called by mapping 1240K or mtDNA capture data to the human mitochondrial reference genome followed by creating pileups at each position (map quality and base quality filter 30) and calling the most frequent base at each position. Resulting genotype information was converted to fasta files and haplogroups were called using haplofind *(87).* Y chromosome haplogroups were called by mapping 1240K capture data to the whole human reference genome (hg19) followed by visual inspection of ancestral/derived alleles (after map quality and base quality filter 30) at ISOGG (v15.58 April 2020) sites.

Principal Components Analysis

Principal components analysis (PCA) was conducted using smartpca (v1600) from the Eigensoft package (https://github.com/DReichLab/EIG). Principal components were calculated on the genotype data of modern West Eurasian individuals (*88–90*) (Table S7). Ancient individuals were projected (lsqproject: YES) onto the axes calculated from modern individuals. "shrinkmode: YES" was used to account for artificial stretching of principal component axes between projected (ancient) individuals and modern individuals. Fst values were also calculated in smartpca using "fsthiprecision:YES" and "inbreed:YES" parameters.

Ancestry decomposition and admixture modelling

F-statistics, qpWave and qpAdm runs were conducted in *admixr* v0.7.1 (*91*), a wrapper program around ADMIXTOOLS (*88*). Selection of outgroups for each analysis is indicated in the corresponding Supplementary Table.
Linear modelling of pre-CW HG ancestry (Figure 3A) was performed using segmented linear regression as implemented in R using the segmented function for v.1.2-0 of the *segmented* library (*92*). To select the optimal number of breakpoints, we compared Akaike's Information Criterion (AIC) for models with between zero and four breakpoints. The AIC is a score that considers how well a model fits the data, while simultaneously penalizing models with additional parameters. In this way, model fit must be significantly improved for a more complicated model with additional parameters to be accepted over a simpler, nested model. A linear regression model with one breakpoint was found to have the minimum AIC, and hence was selected (*93*).

DATES v753(*61*) was used to estimate length distributions of ancestry tracts and infer admixture dates between Anatolia_Neolithic and Western Hunter-Gatherers (WHG, here: Loschbour+Körös_HG+Germany_BDB). Parameters binsize 0.001, maxdis 1, seed 77, jackknife YES, qbin 10, runfit YES, afffit YES, lovalfit 0.45, minparentcount 1 and checkmap YES were used.

Y Haplogroup Frequency Simulations
To investigate the process of Y-haplogroup inheritance in early and late Corded Ware groups, we simulated 10^6 realisations, assuming a generational time of 25 years, and analysed the results using Approximate Bayesian Computation (ABC). For the i^{th} realisation, we assumed a constant population size of $N_i \sim U(10^2,10^4)$, with a starting a proportion of R1a-M417(xZ645) of $p_i \sim TN(0,1)(0.27,0.134)$ from a truncated Normal distribution based on the observed proportion of R1a-M417(xZ645) of 3/11 (0.27). For each simulation we also included a selection coefficient denoted $s_i \sim U(-1,1)$. Under random mating, for generation j+1, let the number of a male offspring carrying R1a-M417(xZ645) be $X_{ij+1} \sim B(N_i,w_j)$, where $w_j = X_{j-1}/N_{j-1}$. However, if one includes a selection coefficient, then $w_j = \min(1,(1+s_i)X_{j-1}/N_{j-1})$. Hence, one may interpret s_j as the average increase in the proportion of male offspring that R1a-M417(xZ645) individuals were having over this period. We then compared our observed number of per generation R1a-M417(xZ645) to our simulated realisations using the rejection method, and keeping the top closest 0.05% realisations (selected via cross validation) to form samples from the joint posterior distributions for our simulation parameters. All ABC and cross-validation analyses were performed in R using the *abc* package (*94*).

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Data Analysis: LP, ABR, SS, WH
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Data Repository of the Max Planck Society (https://edmond.mpdl.mpg.de).

Figures

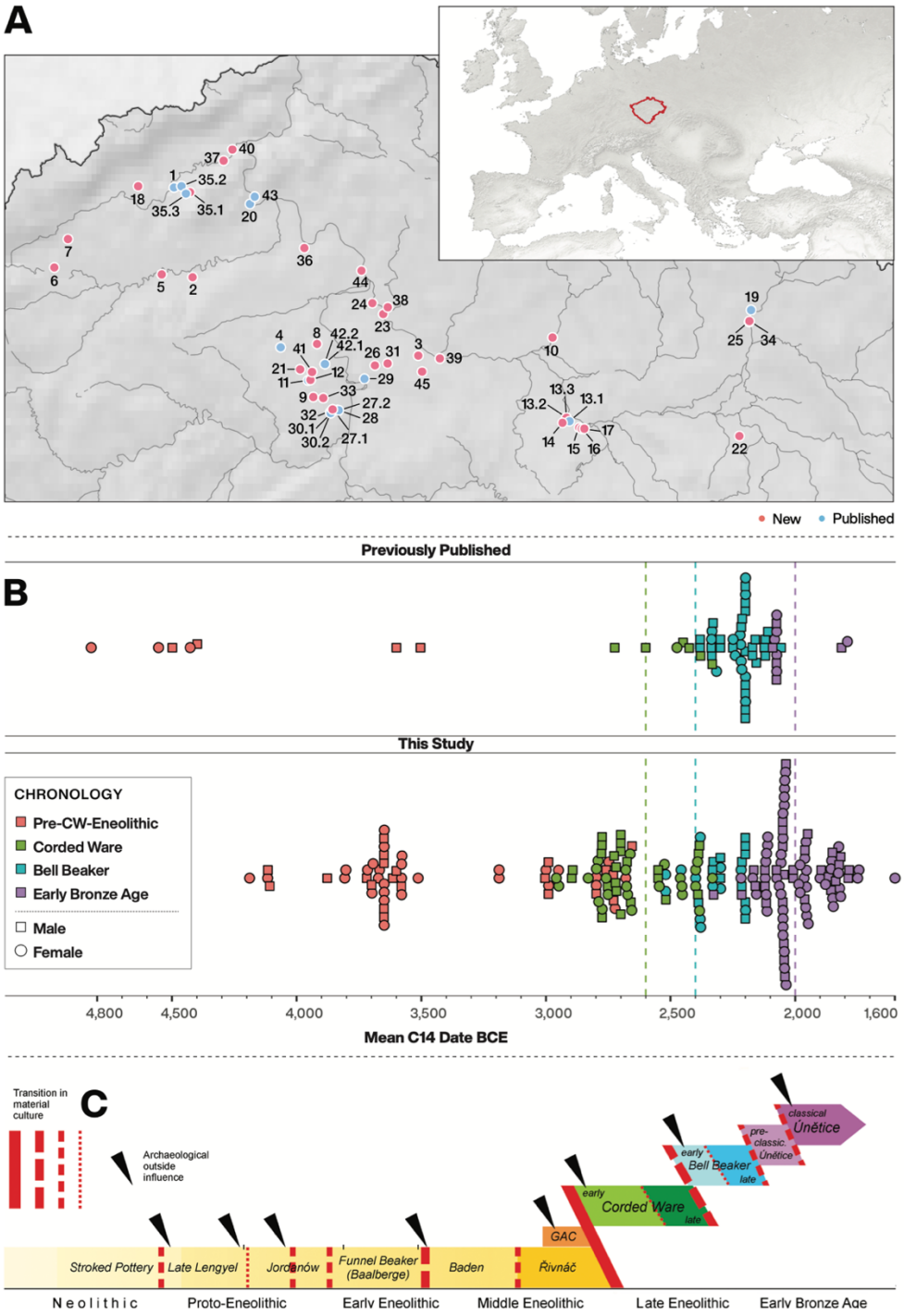

Fig. 1. Temporal and geographic distribution of studied Neolithic, Eneolithic and EBA individuals from Bohemia. A) Map of Bohemia showing the locations of sampled sites (red=new, blue=previously published, Table S2, Fig. S1-S5). **B**) Mean age of newly reported (n=206) and published (n=65) individuals from Bohemia. **C**) Local chronology of archaeological cultures and time periods. Black triangles indicate external influences visible in the material culture. Red lines indicate qualitative degree of change in material culture.

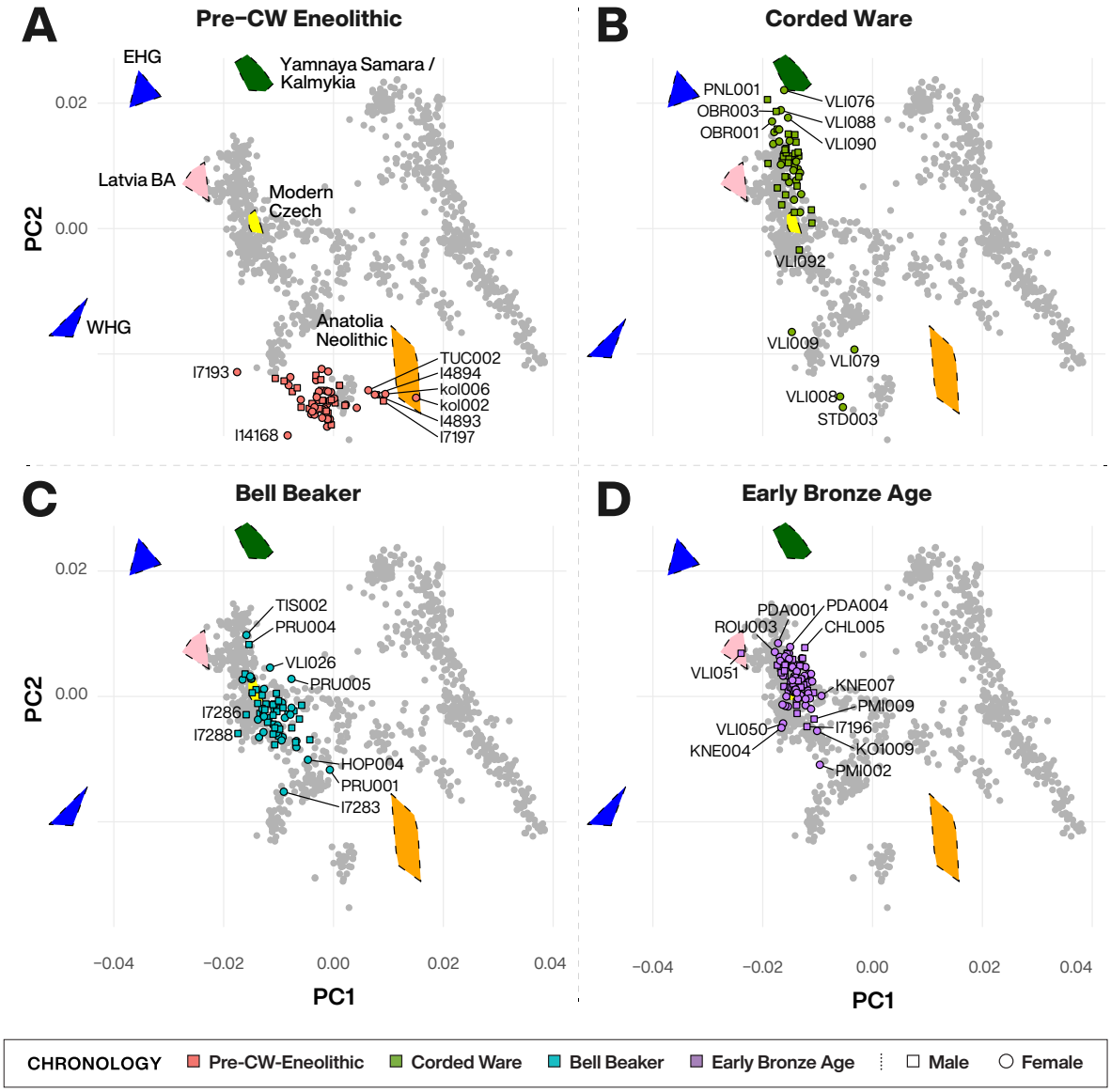

Fig. 2. Principal components analysis (PCA) of published and newly reported ancient individuals from Bohemia (n=271). Data are displayed in four major time periods: (**A**) pre-CW Eneolithic, (**B**) Corded Ware, (**C**) Bell Beaker, and (**D**) Early Bronze Age. Modern-day West Eurasian individuals upon which principal components were calculated (n=1,141, Table S7) are greyed out in the background with modern-day Czech as well as relevant ancients (projected) plotted as colored polygons for reference (labelled in panel A, W(estern)HG, E(astern)HG, Latvia Bronze Age (BA), Yamnaya Samara/Kalmykia, Anatolia Neolithic). Individuals mentioned in the main text are labelled.

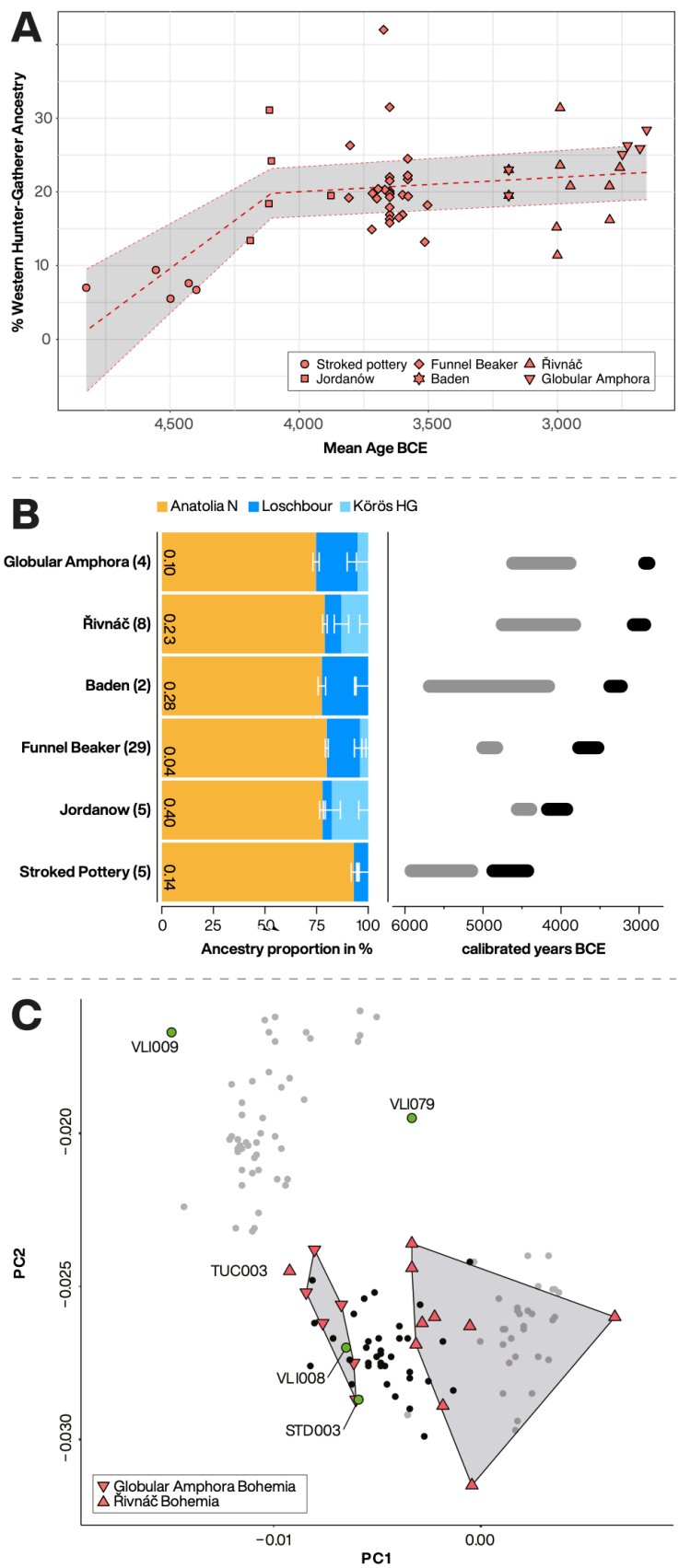

Fig. 3. WHG ancestry in pre-CW Bohemia. (**A**) The proportion of WHG ancestry through time in pre-CW individuals from Bohemia modelled as a two-stage linear process (Table S8). The grey area indicates 95% confidence interval. (**B**) (Left) Proportion of ancestry ascribable to Anatolia_Neolithic, Loschbour and Körös_HG in pre-CW cultural groups from Bohemia in chronological order from bottom to top (Table S10, sample size of each cultural group in brackets and p-value of three-way *qpAdm* model indicated within orange bars). (Right) Inferred dates (Table S11, 2 standard errors) of HG admixture (grey interval) relative to culture's chronology (black interval). (**C**) Zoomed-in PCA showing (with the exception of TUC003) segregation between Bohemian GAC and Řivnáč individuals along with position of early CW females without "steppe" ancestry (green circles). Black dots represent previously published GAC individuals from Poland and Ukraine.

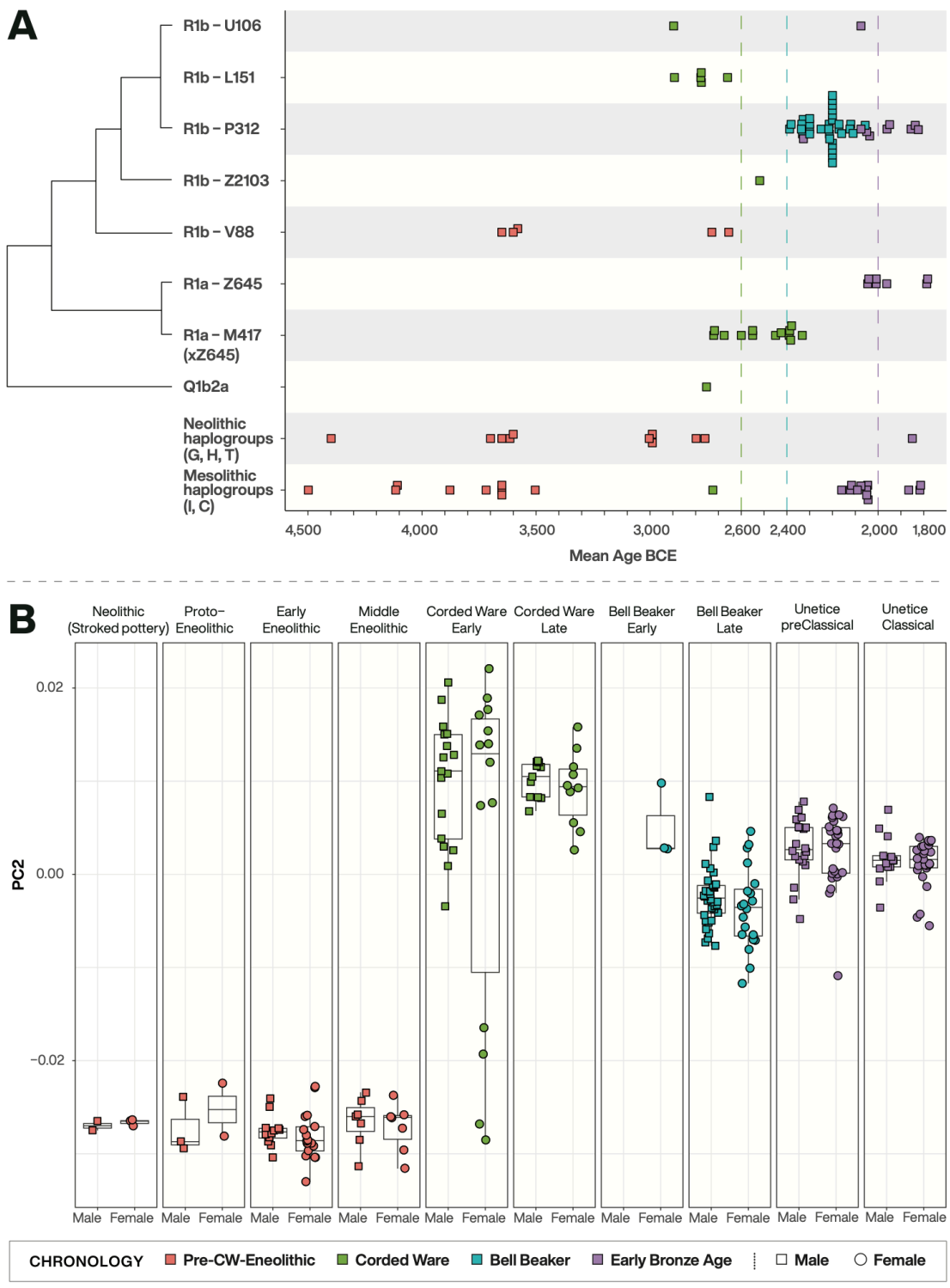

Fig. 4. Temporal Y-chromosome and autosomal PC2 variation in Bohemia. (A) Temporal distribution of Y chromosome haplogroups by culture. Schematic of phylogenetic relationships between Y-chromosome lineages shown along y-axis. Dashed vertical lines demarcate respective (colored) cultural group into early and late phases. (B) Temporal variation in PC2 showing the genetic variation of males and females within each cultural group.

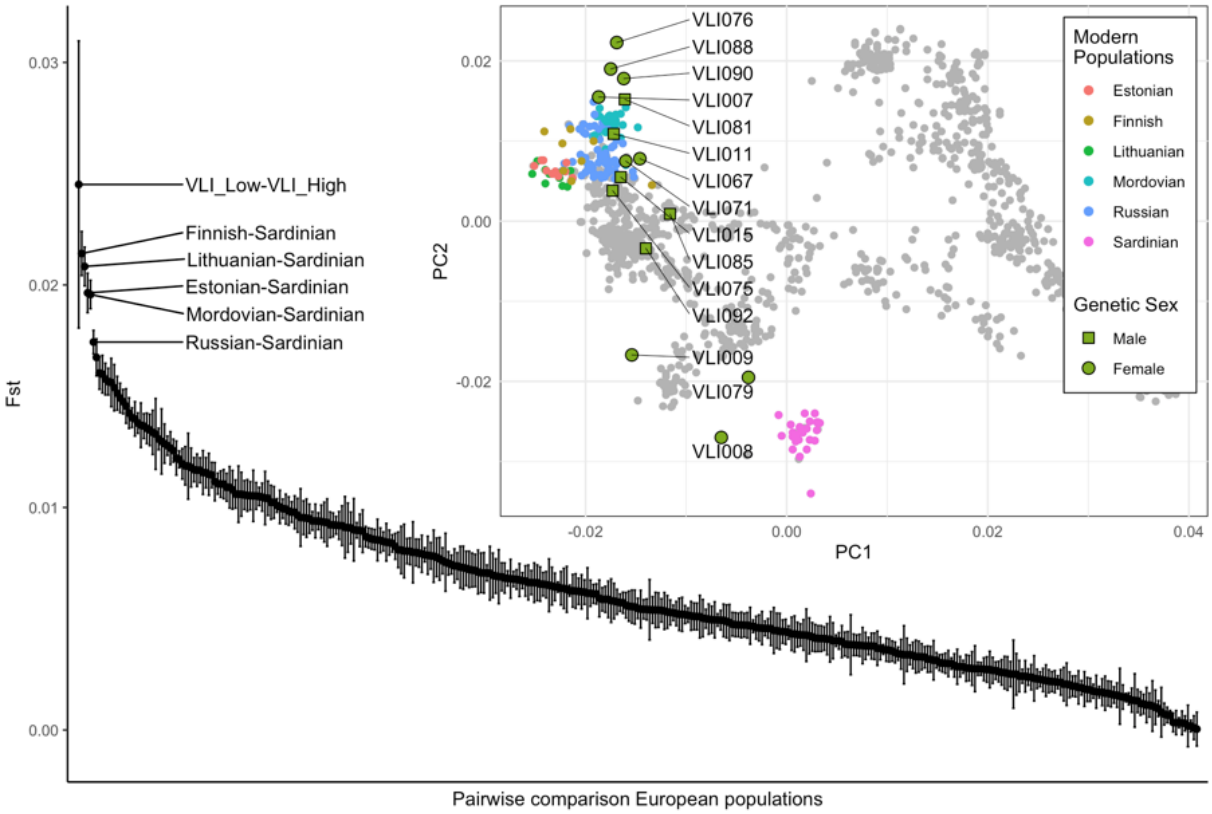

Fig. 5. Genetic distances of Early Corded Ware individuals from Vliněves. Pairwise F_{st} between three highest (VLI076, VLI088, VLI090; VLI_High) and three lowest (VLI008, VLI079, VLI009; VLI_Low) early CW on PC2 from Vliněves in the context of modern European pairwise F_{st} (2 standard errors plotted, Table S27). (Inset) PCA of 1141 modern West Eurasian individuals (grey points) on which early CW individuals from Vliněves (green symbols with black outline) were projected (see also Fig. 2B). The highly differentiated pairs of modern European populations labeled in the main figure are colored in the inset PCA.

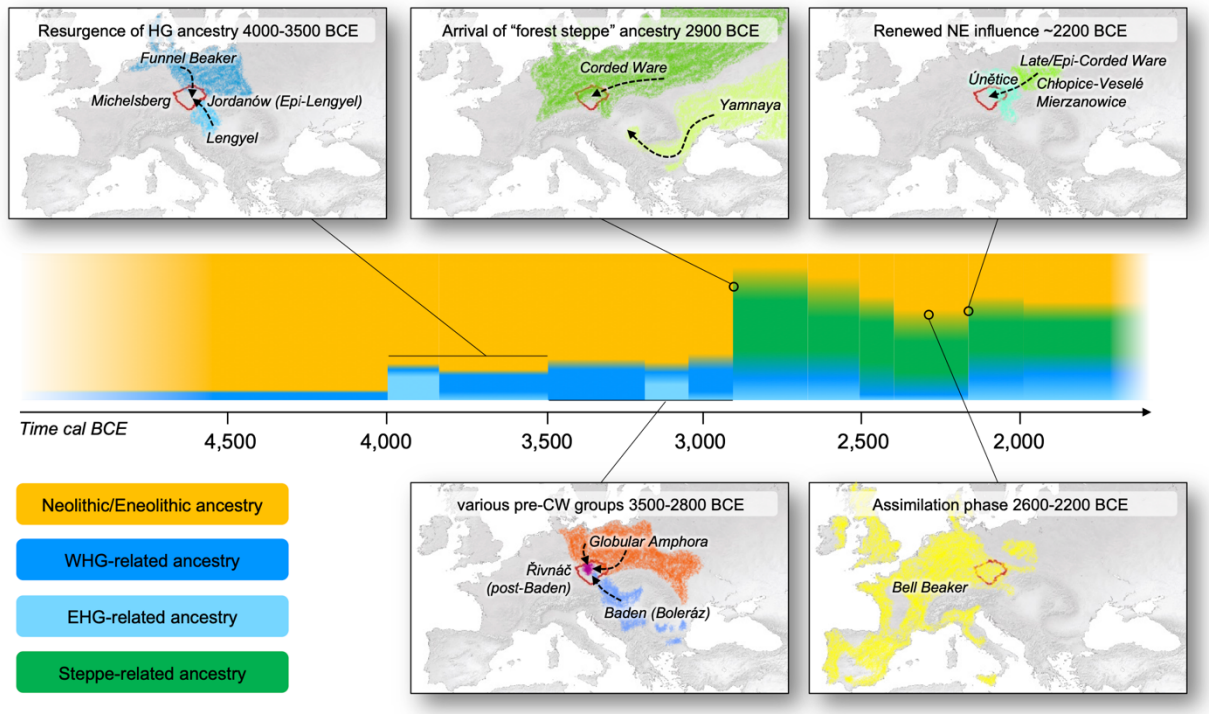

Fig. 6. Schematic summary of the major processes that shaped the genetic and cultural diversity of Bohemia (red outline) over time. Arrows on maps indicate a general direction of influences rather than discrete routes of migration.

Supplementary Materials

Supplementary Text – Archaeological background, C14 dating and sites descriptions

Figs. S1 to S9

Tables S1 to S38 (separate Excel spreadsheets burnt onto CD which accompanies this thesis)

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Supplementary Materials for Manuscript A.

Dynamic changes in genomic and social structures in 3rd millennium BCE central Europe

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This PDF file includes:

Supplementary Text
Figs. S1 to S9
Table S37
Captions for Tables S1 to S36 (separate Excel spreadsheets have been burnt onto CD which accompanies this thesis)

Other Supplementary Materials for this manuscript include the following:

Tables S1 to S36 (separate Excel spreadsheets burnt onto CD which accompanies this thesis)

Archaeological background

While this study is devoted primarily to archaeogenetic data it is important to briefly summarise the archaeological background and context of the presented datasets and research questions, namely in the „old fashioned“ and maybe „long-outdated“ (*38*) but still broadly accepted culture-historical way of “archaeological cultures“ (Table S38, Fig. 1; see the last handbooks to Bohemian prehistory (*49*, *50*, *59*)), by understanding them actually rather as “archaeological units of classification” (mainly of artefact styles, burial practices etc.) than in the sense of recently rightly criticised „distinct groups of people“ (*21*, *33*, *37*, *38*, *40*, *95*, *96*).

The region of focus concerns the northern part of Bohemia, the basins of the Elbe, lower Vltava and Ohře rivers and the Bohemian part of the Ore Mountains. South and west Bohemia were not settled densely before the EBA. Before the Neolithic, these mainly forested regions at higher elevations were occupied by late Mesolithic hunter-gatherer groups, who may have persisted for some time during the Eneolithic (*97*).

The Bohemian Stone Age prehistory is divided into two basic epochs: the Neolithic (ca. 5400–4400 BC) and Eneolithic (ca. 4400–2200 BC). The Neolithic is represented by the Linear Pottery (LBK, in Bohemia ca. 5400/5300–5000 BC) and Stroked Pottery cultures (STK, ca. 5000–4400 BC). There is broad consensus that the STK was derived from the LBK, without influence from outside (*49*).

The emergence of the Lengyel culture (ca. 4400–4200 BCE) (*49*) is regarded as a culture-historical turning point and marks the beginning of the Eneolithic in Bohemia (*50*) triggered by the arrival of a new population from the southeast (Moravia, Austria, Pannonia, southwest Slovakia). The Jordanów culture (ca. 4200–3900 BC) is also included in the initial proto-Eneolithic period. Although culturally tied to the preceding Lengyel development, elements from the western Michelsberg culture are strongly manifested in the later phase. The status of the Michelsberg culture in prehistoric Bohemia is unclear, as Bohemia is on the boundary of two cultural traditions/phenomenons, the eastern Lengyel and western Michelsberg. Consequently, some scholars considered Michelsberg an autonomous entity, others a foreign influence into local Jordanów and older Funnel Beaker (Baalberge) culture (*98*, *99*). From Jordanów/Michelsberg contexts exist first evidence of burials under barrows (Březno u Loun (*100*)), assumed also for the Funnel Beaker period and later on a mass scale for the CW and BB (*50*), alternatively for the EBA (*101*).

The Early Eneolithic (ca. 3800–3400 BC) is represented by the Funnel Beaker culture (Baalberge, incl. Siřem-stage). More than one hundred single inhumation burials in a crouched position and tens of burials in settlement features are recorded. The single graves with skeletons in a crouched position are characteristic since neolithic (LBK, STK) and as such for the entire Bohemian Eneolithic and EBA. Collective graves, which are typical for the Funnel Beaker in northern Europe, are absent in Bohemia completely.

The Middle Eneolithic (ca. 3400–2800 BC) was a period of cultures associated with the Baden cultural complex. The earliest stage of Baden in Bohemia (Boleráz) is thought to present a new population from the core of the Baden cultural complex in Carpathian Basin (*102*). In the following horizon, the late Funnel Beaker culture (Salzmünde (*103*)) is replaced by the classic Baden culture, from which the local post-Baden cultures develop: Řivnáč in central and northwest Bohemia, Bošáca in east Bohemia and Cham in west Bohemia (all ca. 3100–2800

BC). Inhumation graves during this period were quite rare (e.g. Holubice in this study) and the available anthropological material comes mainly from settlement features (sunken pits, semi-sunken huts etc.).

The Globular Amphora culture (GAC) extended into Bohemia as a new entity during the final Middle Eneolithic and its bearers are unanimously regarded as newcomers from the north. The GAC was partially contemporaneous with post-Baden Řivnáč and Cham cultures (GAC pottery was repeatedly found in settlements of both) (*56*) and is manifested by few burials of individuals in a crouched position. Regarding the possible coexistence of Řivnáč and GAC in Bohemia two possible scenarios were discussed. Firstly, the contemporaneous occupation by exploitation of different territories by more or less complete replacement of the Řivnáč settlement by the GAC in the late phase, secondly infiltration of GAC-people into the Řivnáč society (*56*, *104*).

A distinct turning point in cultural development was the emergence of Late Eneolithic Beaker phenomena: Corded Ware (CW; ca. 2900/2800–2400 BC) and Bell Beaker (BB; ca. 2500–2200 BC). Both had a large geographic distribution in Europe, with the CW in central and NE Europe and BB in central, north- and southwestern and southern Europe. The CW in Bohemia is almost exclusively limited to grave finds with skeletons in a crouched position in W-E orientation with females on their left side, and males on their right side. While the number of investigated graves is one of the highest in Bohemian prehistory (ca. 1,500 graves), human skeletal material has not been preserved in all of them. Views on the origin of the CW differed greatly, from migration models (*57*, *105*) to a purely autochthonous emergence (*106*), as did opinions on the subsistence, which ranged from a culture of settled farmers (*107*), to a pastoral nomadic character (*108*). The CW in Bohemia was not uniform over time, and three phases can be distinguished archaeologically: early (A-horizon, Kalbsrieth-type graves), middle ("Fischgrätenbecherhorizont") and late (local Bohemian Corded Ware) – material groups 1 – 3 after M. Buchvaldek (*109*).

The Bell Beaker phenomenon (BB) in Bohemia is represented by hundreds of documented inhumation and cremation burials (ca 10 %). The inhumation ritual stands in contrast to the Corded Ware with males mostly in a left-crouched position, and females mainly in a right-crouched position, in N-S orientation. Various interpretations exist about the origin of the BB in Bohemia, both allochthonous (Iberian Peninsula Northern Africa, Lower Rhine Region, etc.) and autochthonous, with advocates of both theories in Czech archaeology (*58*, *110*, *111*). A typo-chronology of BB should be compiled from graves containing decorated beakers (early stage) towards graves with so-called "associated pottery" – late stage. In Bohemia, this so-called "associated pottery" ("Begleitkeramik") is very similar to the pottery of the early phase of the EBA Únětice culture, which has been interpreted as evidence of continuity in material culture between the two.

The central European EBA is characterised by the so-called Únětice culture, mostly known from thousands of inhumation graves in a N-S-oriented, right-crouched position facing east and with no apparent gender differentiation in orientation (unlike the CW and BB). Bohemia can be considered its core area. Traditionally it is separated into two main parts: early (proto-Únětice and pre-classic phases) and late (classic to post-classic) phases after ~2000 BCE (*59*, *112*, *113*). The late (classical) phase is characterised by large hoard finds, typical Únětice cups, eyelet pins (Ösenkopfnadeln) and large cemeteries with inhumation burials rich in bronze artefacts, amber and gold jewellery and other exotics (*60*, *75*, *77*, *114–117*). There is no

continuity at many cemetery sites from the early to the late phase. Early Únětice grave groups are smaller (mostly less than 10–15 graves), graves contain almost exclusively vessels, and only rarely copper wire artefacts.

The only one Middle Bronze Age (MBA) individual which we have incorporated in our study is that from the only one burial of this age from the important site Vliněves, grave 504 (VLI053), containing female skeleton in age of 50+ years buried with two typical MBA bronze pins. In the qpAdm modelling we group this skeleton with Bohemia_Unetice_Classical samples.

Table S38. Chronological framework of the periods and archaeological cultures discussed in the text.

Period	Archaeological culture	Phase	cal. BC
N e o l i t h i c	Linear Pottery (LBK; Linearbandkeramik)		5400/5300-5000/4900
	Stroked Pottery (STK; Stichbandkeramik)		5000/4900-4400/4300
E n e o l i t h i c	Lengyel		4400/4300-4300/4200
	Jordanów / early Michelsberg		4300/4200-3900/3800
	late Michelsberg / Funnel Beaker		3900/3800-3500/3400
	Baden		3500/3400-3200/3100
	Řivnáč / Cham / Bošáca		3200/3100-2900/2800
	Globular Amphora		3000/2900-2900/2800
	Corded Ware		2900/2800-2500/2400
	Bell Beaker		2500/2400-2200/2100
E B A	Únětice (Aunjetitz)	early	2300/2200-2000/1950
		late	2000/1950-1750/1700

Sampled sites in Bohemia

Site numbers correspond to numbers on the maps (Figures S1-S5) and in Table S2.

blue – published in (*4*, *5*, *61*, *62*)

1. Bílina – published (*61*)
2. Blšany
3. Brandýs nad Labem
4. Brandýsek – published (*5*)
5. Březno u Loun
6. Čachovice
7. Droužkovice
8. Holubice
9. Hostivice
10. Chleby
11. Kněževes 1 – published (*4*, *5*)
12. Kněževes 2

13–16. Kolín, road bypass, Sites I, II, VI and VII

13.1. Kolín, road bypass, Site I-3 – published (*62*)
13.2. Kolín, road bypass, Site I-7a – published (*62*)
13.3. Kolín, road bypass, Site I-7b
14. Kolín, road bypass, Site II
15. Kolín, road bypass, Site VI - Polepy
16. Kolín, road bypass, Site VII
17. Kolín - Šťáralka
18. Konobrže
19. Lochenice – published (*4*, *5*)
20. Lovosice – published (*4*, *5*)
21. Makotřasy
22. Mikulovice
23. Neratovice
24. Obříství
25. Plotiště nad Labem
26. Praha - Ďáblice
27.1. Praha - Jinonice
27.2. Praha - Jinonice, Holman's garden centre – published (*5*)
28. Praha - Jinonice, Butovická St. – published (*5*)
29. Praha - Kobylisy, Ke Stírce St. – published (*5*)
30. Praha - Malá Ohrada
30.1. Praha - Malá Ohrada (CW)
30.2. Praha - Malá Ohrada (BB) – published (*5*)
31. Praha - Miškovice
32. Praha - Nové Butovice
33. Praha - Ruzyně
34. Předměřice nad Labem
35.1. Radovesice
35.2. Radovesice – published (*5*)
35.3. Radovesice – published (*61*)

36. Roudnice nad Labem
37. Stadice
38. Tišice
39. Toušeň
40. Trmice
41. Tuchoměřice
42.1. Velké Přílepy
42.2. Velké Přílepy – published (*4*, *5*)
43. Velké Žernoseky – published (*61*)
44. Vliněves
44.1. Vliněves – Eneolithic (pre-CW)
44.2. Vliněves – Corded Ware
44.3. Vliněves – Bell Beaker
44.4. Vliněves – Early Bronze Age
45. Zeleneč

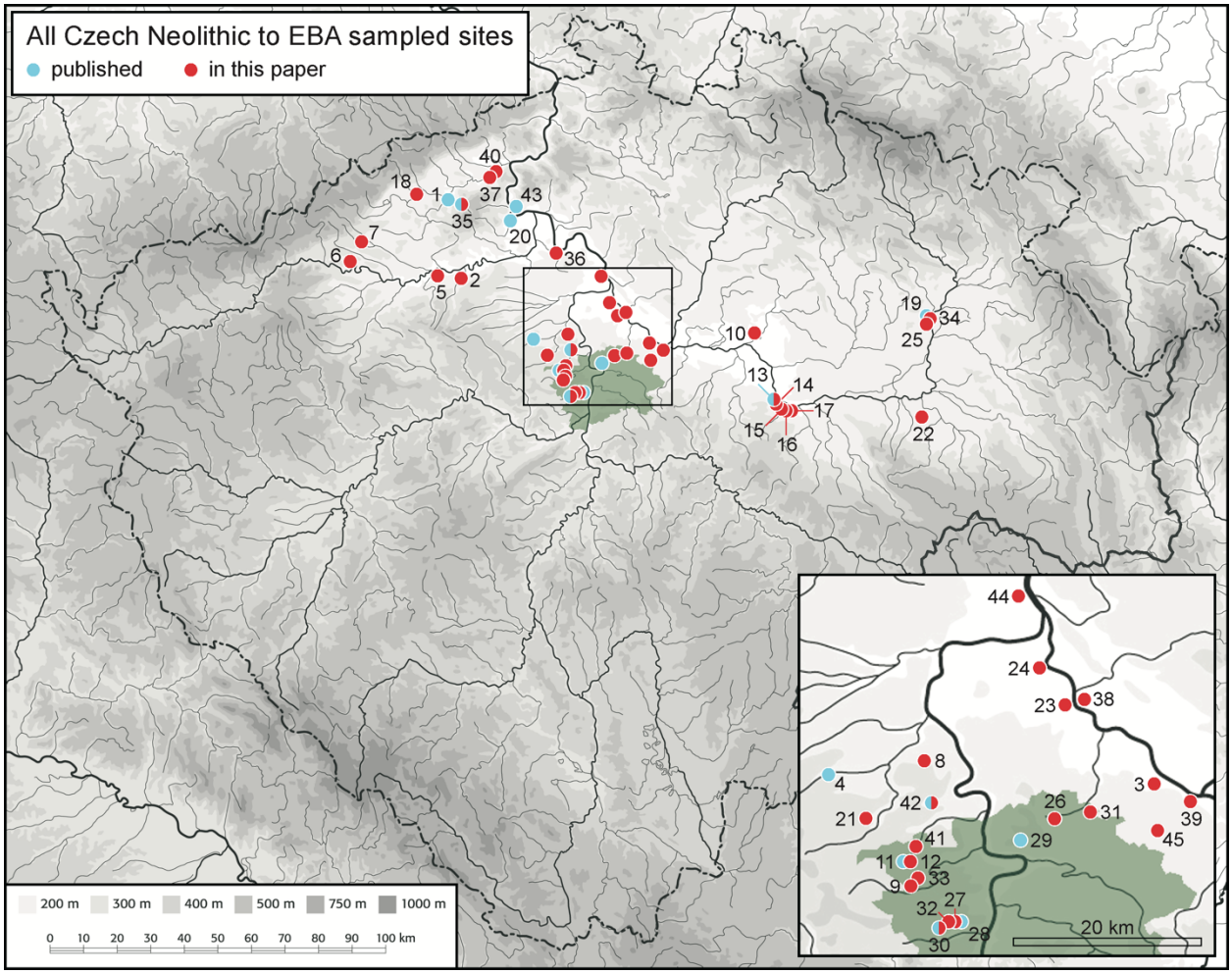

Fig. S1. All Neolithic to EBA sampled sites. Site numbers correspond to site names listed above and in the site descriptions (Table S2). Green: Metropolitan area of Prague (capital city). Graphic M. Ernée, M. Dobeš.

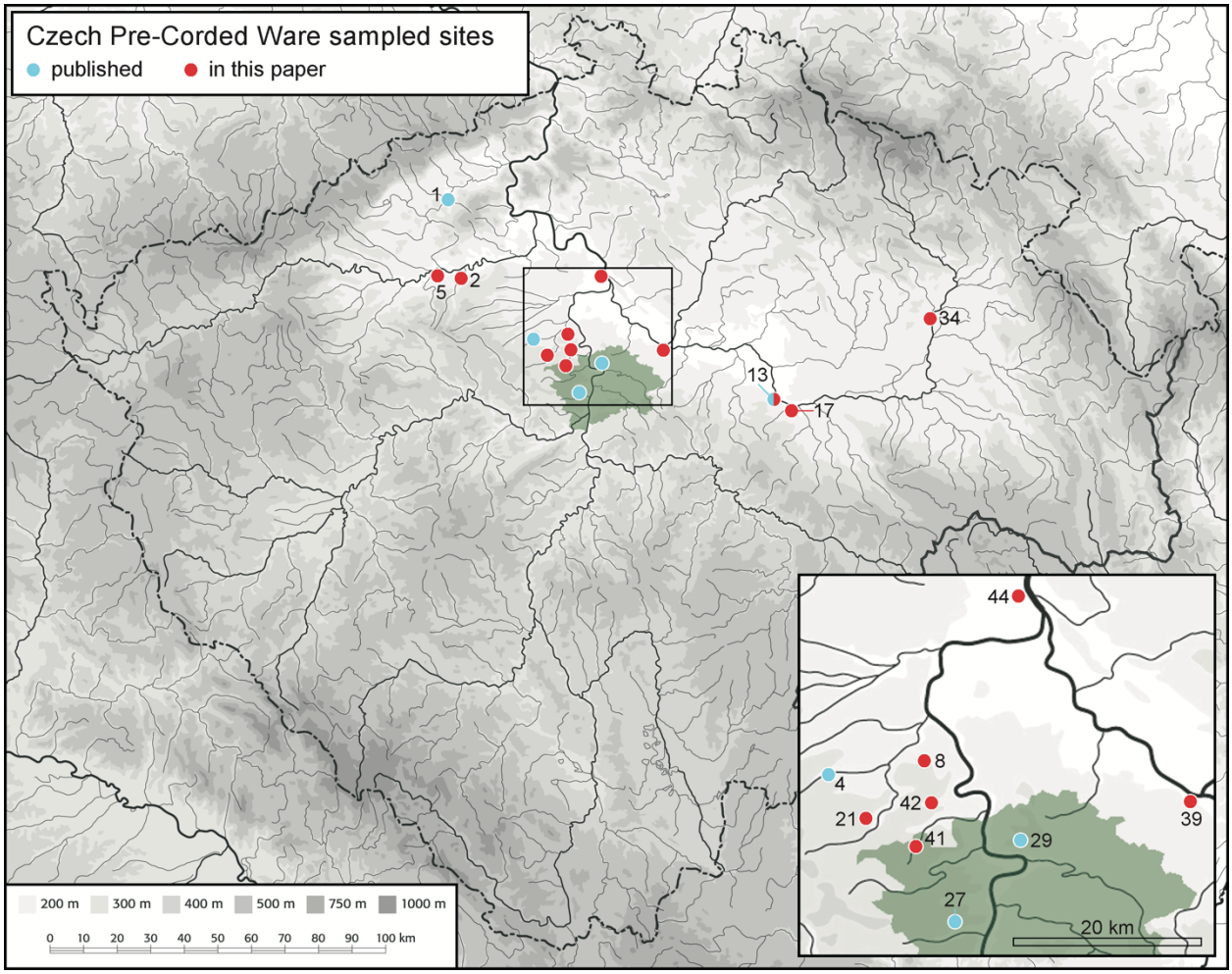

Fig. S2. Pre-Corded Ware sampled sites. Site numbers correspond to site names listed above and in the site descriptions (Table S2). Green: Metropolitan area of Prague (capital city). Graphic M. Ernée, M. Dobeš.

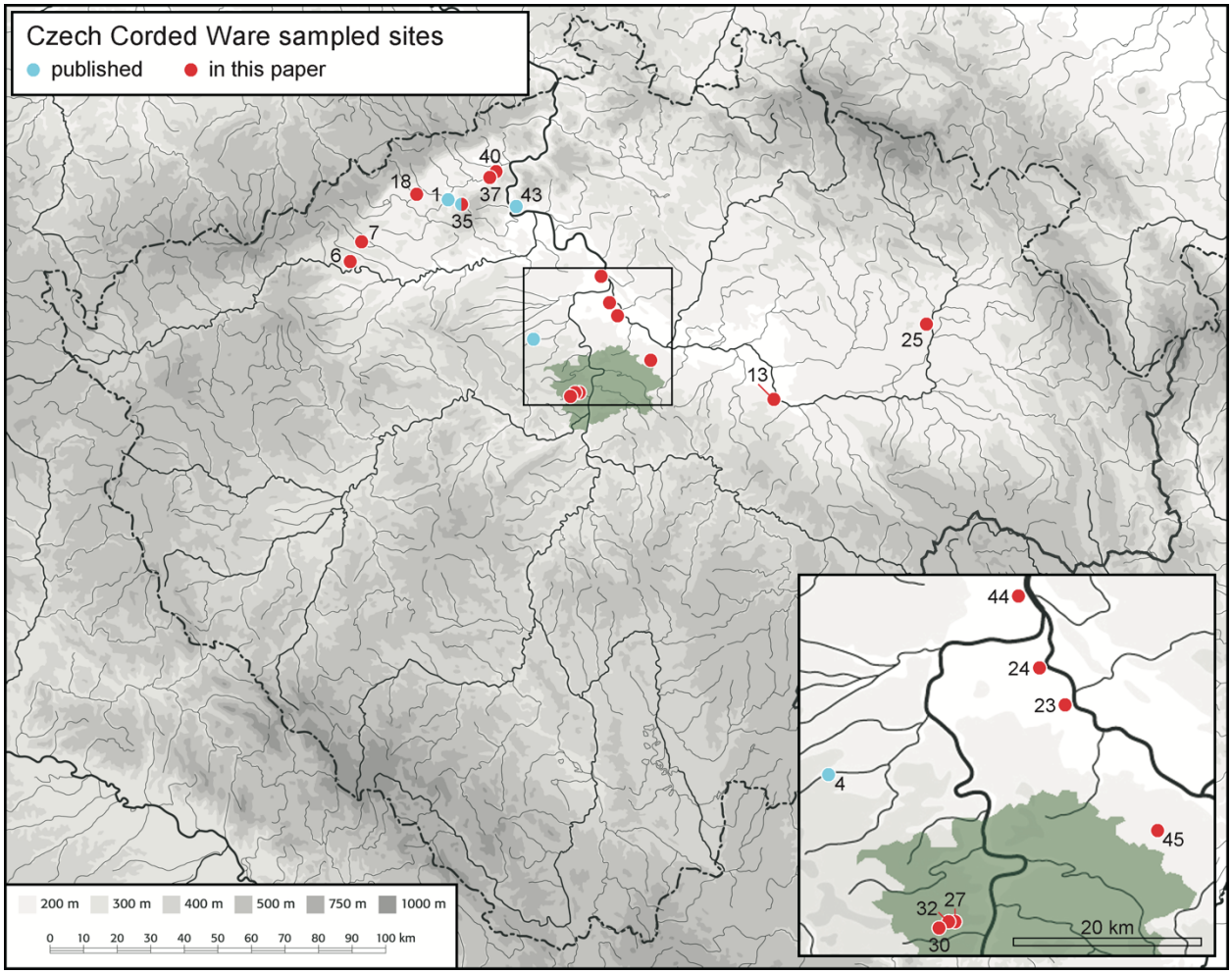

Fig. S3. Corded Ware sampled sites. Site numbers correspond to site names listed above and in the site descriptions (Table S2). Green: Metropolitan area of Prague (capital city). Graphic M. Ernée, M. Dobeš.

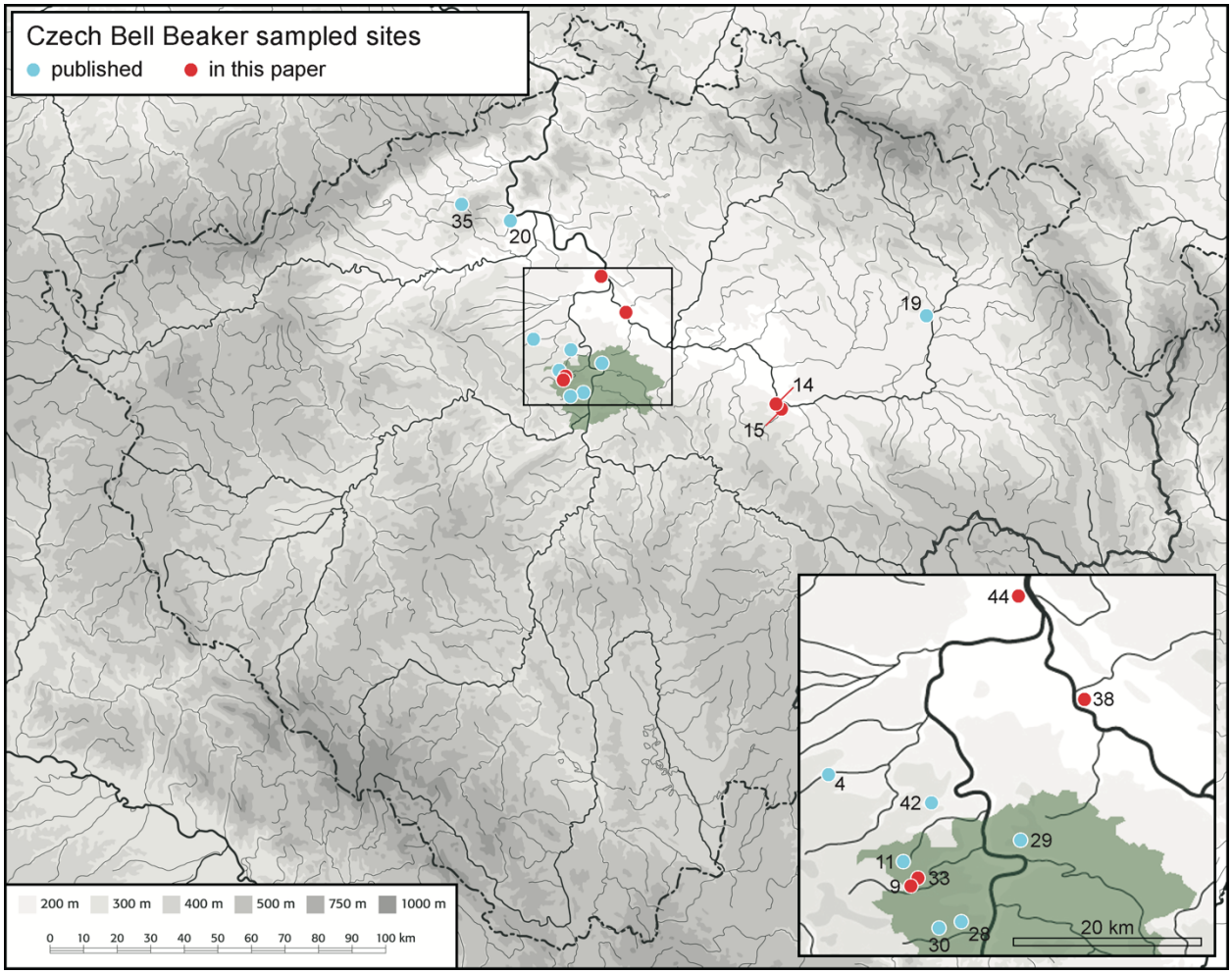

Fig. S4. Bell Beaker sampled sites. Site numbers correspond to site names listed above and in the site descriptions (Table S2). Green: Metropolitan area of Prague (capital city). Graphic M. Ernée, M. Dobeš.

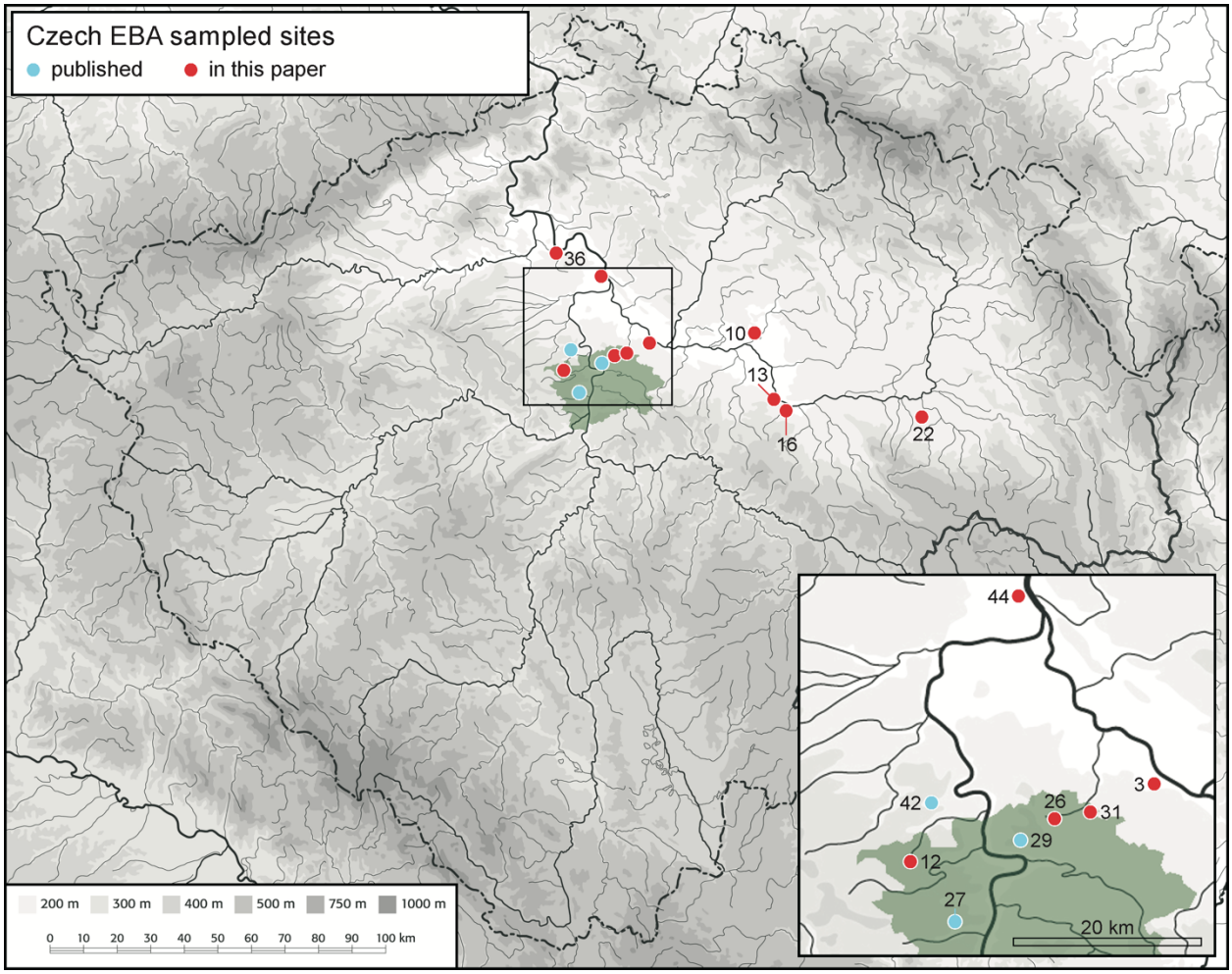

Fig. S5. EBA sampled sites. Site numbers correspond to site names listed above and in the site descriptions (Table S2). Green: Metropolitan area of Prague (capital city). Graphic M. Ernée, M. Dobeš.

Radiocarbon dating

In this study we report 230 radiocarbon data in total from 197 skeletons found in 178 graves or settlement sunken features. We include previously published (n=90) as well as new data (n=140) generated in 12 different radiocarbon laboratories (Table S6):

- Curt-Engelholm-Zentrum Archaeometrie (MAMS) – 116
- Pensylvania State University (PSUAMS) – 39
- Czech Radiocarbon Laboratory (CRL) – 32
- Leibnitz-Labor Kiel (KIA) – 11 and (KI) – 3
- Centre for Climate, the Enviroment and Chronology, Queen´s University of Belfast (UBA) – 10
- Utrecht van de Graaff Laboratorium (UtC) – 6
- Centre for Applied Isotope Studies, The University of Georgia (UGAMS) – 5
- Centre for Isotope Research, University of Groningen (GrN) – 3
- Poznań Radiocarbon Laboratory (Poz) – 3
- Bristol Radiocarbon Accelerator Mass Spectrometer (BRAMS) – 1
- Berlin (Bln) – 1

The 197 dated skeletons date to the Neolithic (n=5), preCW Eneolithic (n=33), CW (n=51), BB (n=37) and the EBA (n=71; 32 of them to the early and 39 to the late/classical stage of the Únětice culture). For Řivnáč, GAC, CW, BB and both stages of the EBA Únětice culture (AK1 and AK2) the 14C data were modelled to create time-intervals of duration of these cultural phenomena (Fig. S8).

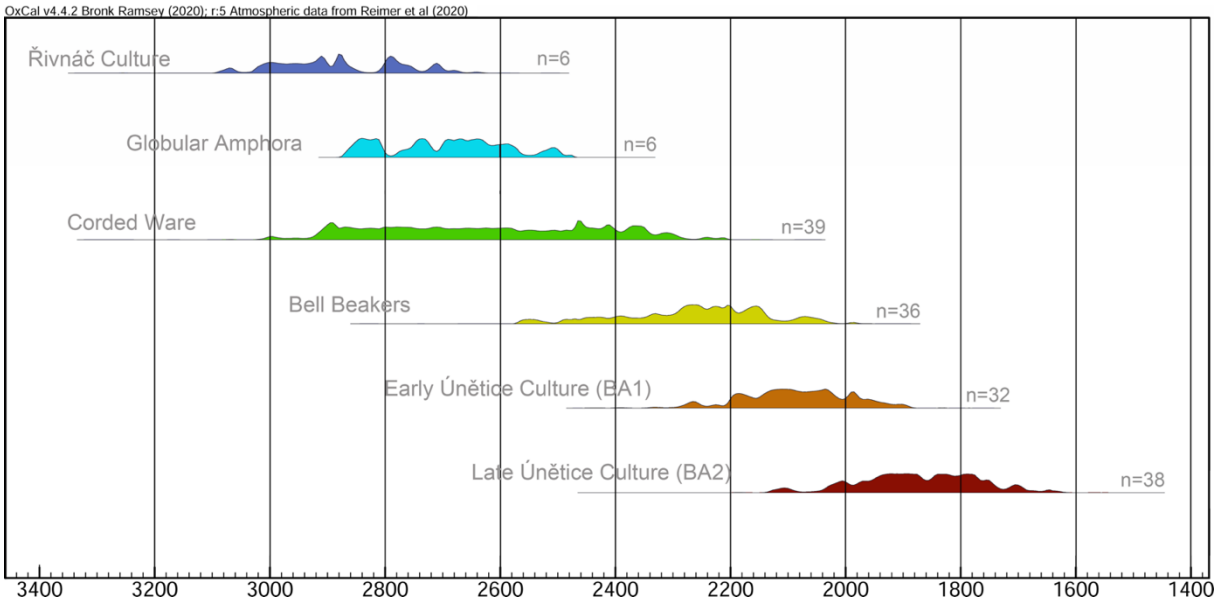

Fig. S8. AMS 14C intervals. Modelled intervals for Řivnáč, GAC, CW, BB and EBA early (pre-classical stages of the Únětice Culture) to EBA late (classical to post-classical phase of the Únětice Culture) AMS 14C data from Bohemia used in this paper. n = number of modelled radiocarbon dates. Graphic M. Langová.

In Table S6 we have collated all available 14C data relevant to individuals/graves represented in this study, including from human bones (n=220), animal bones (n=9) macro remains (n=1), and older (no AMS) as well as newer ones (AMS). For the sake of completeness, we also list samples that contained low ratios or no collagen (n=5), and one sample which was lost during the procedure. Table S6 also includes all supporting data such as δ13C, C:N, C /%/, collagen /%/ and bone specifications where available.

In cases where dates from human and animal bones were available from the same grave, we used the date from human bones. Where both AMS and no-AMS data were available from the same skeleton, we used the AMS date. Dates that were not used (from animal bones or no-AMS data) are highlighted in grey. These were only taken into consideration when it was the only available data from the individual grave/skeleton. Where more than one AMS date from the same skeleton was available, we used the modelled mean value.

On the basis of the new results, we briefly comment on dates from three graves:

21. Makotřasy, settlement pit 51/61. From this settlement feature three 14C data were produced, one from human bone (PSUAMS-4404, 4228–3988 2-sigma cal BCE) and two earlier non-AMS dates from animal bones (GrN-6928, 3623–2926 2sigma cal BCE; GrN-6929, 3635–3371 2-sigma cal BCE). The date from the human bone is significantly older than both earlier dates from animal bones. Although the dates from both animal bones would fit much better to the finds from the pit filling and also compared with the data from other skeletons from this site, we accept here the direct date from the human bones.

24. Obříství, grave 1. In grave 1 a female and a newborn child were buried (based on aDNA kinship analyses a first-degree mother/child relationship). Based on the first AMS dates of both individuals (MAMS-30794 – 4276 ±22 BP and MAMS-38471 – 3941 ±25 BP), the date from the newborn was significantly younger than the date from the mother (interval of 335 years BP) and the calibrated 2-sigma intervals showed no overlap (Fig. S9: green). Hence, we commissioned additional dates from both skeletons, in this case directly from the both petrous bones, from where aDNA was sampled, which documented the first-degree relatedness between both. However, also the second series of dates (MAMS-41363 and MAMS-41364) showed no overlap of the 2-sigma intervals (Fig. S9: orange). In this case the date from the newborn (3861 ±34 BP) was also younger than the date from the mother (4064 ±23 BP). For each skeleton we have modelled the mean value of both 14C data. The resulting 2-sigma intervals for the mother (2881–2674 cal BCE) and the child (2469–2305 cal BCE) show also no overlap. The fifth 14C AMS date was produced from a decorated bone disc found with the mother (BRAMS-2959) and shows a 2-sigma interval of 2617–2467 cal BCE. At this stage, we have not reached a final interpretation of the 14C data from this grave complex other than an in-depth evaluation of all lines of evidence will be necessary, especially since both dates from the mother (MAMS-30794 and MAMS-41363) have failed a chi-squared test with a less than 5% chance this being a good combination.

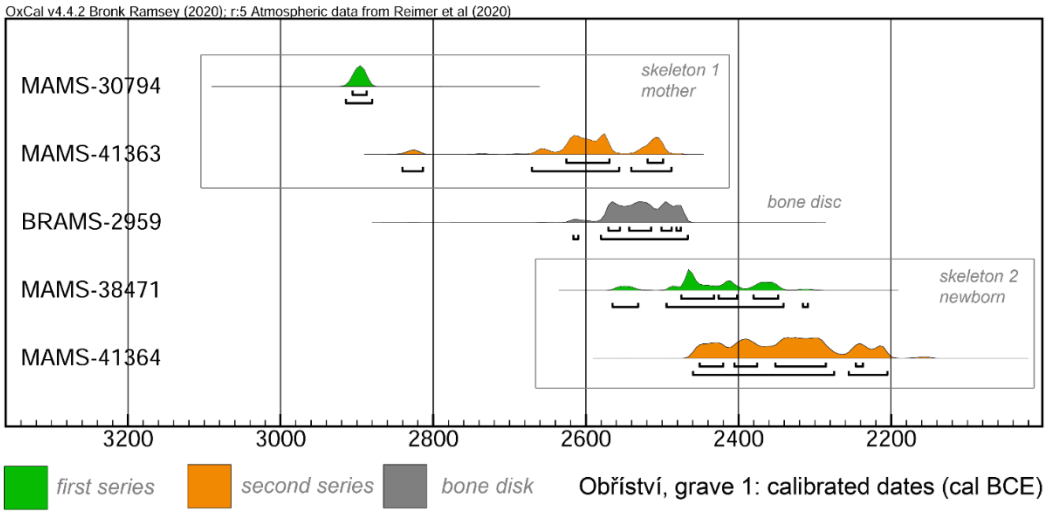

Fig. S9. Radiocarbon dates of the CW grave 1 from Obříství. Graphic M. Langová.

44.2. Vliněves, grave 5790. The first 14C wider date (CRL-9201; 2861–2148 2-sigma cal BCE) was not generated with AMS. The newly created AMS (MAMS-41359; 2286–2138 2-sigma cal BCE) results dating to the EBA is considered too young/late in our opinion, as the grave is more in line with the CW period according to position of the body and the grave goods (upper part of big amphora). Thus, a third sample was taken directly from the petrous bone, which was sampled for aDNA. This produced a date (MAMS-46362; 2473–2311 2sigma cal BC), which is in line with the archaeological context and the genetic results.

Detailed site descriptions and anthropological information

1. Bílina, Titzler sandpit (Bílina, Teplice district, NW-Bohemia, Czech Republic)

Published in (61)

The site was being destroyed by sand pitting for a long time and a large number of graves from the Eneolithic up to the Early Middle Ages have been documented. However, many other graves have been destroyed without any evidence. Eneolithic graves were mostly uncovered at the beginning of the 20th century, when little attention was usually paid to anthropological material; therefore, any human remains rarely appeared in museum collections (*118*). The original number of Eneolithic graves on this site can be estimated at several dozen (*119*).

Grave from 1903. The skeleton was delivered along with three vessels belonging to the middle or late stage of the Corded Ware culture; the dating of skeletal material is, therefore, not very reliable. Only the skull has been preserved. Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: adultus (30–40?). Grave goods: amphora, jug, small pot (potentially, there might have been more vessels). Archaeological dating: Corded Ware culture, middle/late stage. Radiocarbon dating: not available *(61, 118, 119)*. Master ID and/or other aDNA signs: I6695, BILI_139. NM Prague Inv. No.: P7A 7564.

Grave from 1910. Two skeletons in crouched(?) position – a man and a woman – lying probably in a grave next to each other, in east-west orientation. Only the male skeleton (I6677; P7A 7558) has been analysed. Sex: archaeology – ?, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: decorated amphora. Archaeological dating: Funnel Beaker culture (late Baalberge). Radiocarbon dating: not available*(61, 118, 120)*. Master ID and/or other aDNA signs: I6677, BILI_4. NM Prague Inv. No.: P7A 7558.

2. Blšany (Blšany u Loun, Louny district, NW Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský

An inhumation burial was investigated in the wall of the sandpit in 1957 (J. Šimůnek). The grave was entirely preserved; only its northern part was slightly disturbed by mining activities. In addition to the grave goods described below, the grave fill contained finds of settlement character (potsherds, animal bones, fragment of grinder stones, etc.), too. Among them, pottery of the Globular Amphora culture was identified. Although the skeleton originally identified as an adult male (see *(121)*, based on skull traits), pelvic traits identified by modern investigation suggest it probably belongs to a female individual (*P. Velemínský*) which is consistent with the results of the aDNA analysis. The grave was published in full in *(122, 123)*.

Grave 1. Grave with two burials

Skeleton 1: right-sided crouched burial, head towards the north. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: adult (20–40?). Grave goods: two-handled amphora, four-handled amphora, cup, pot, fragments of other vessels. Archaeological dating: Globular Amphora culture, western group. Radiocarbon dating: MAMS-41369 (4126±24) 2865–2583 cal BC 2-sigma *(121-123).* Pandora No.: BLS001. NM Prague Inv. No.: P7A 31831A.

Skeleton 2: left-sided crouched(?) burial, head towards the north. Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: fetus or newborn. Grave goods: see skeleton 1. Archaeological dating: Globular Amphora culture, western group. Radiocarbon dating: MAMS-41370 (4141±22) 2871–2627 cal BC 2-sigma *(121-123).* Pandora No.: BLS002. NM Prague Inv. No.: P7A 31831B.

3. Brandýs nad Labem (Brandýs nad Labem – Vrábí, Prague-East district, central Bohemia, Czech Republic)

Contact persons: Michaela Langová, Alžběta Danielisová, Petra Stránská

The enclosed Early Bronze Age (BA2) settlement site in Brandýs nad Labem - Vrábí has been excavated by A. Danielisová, M. Pecinovská, K. Čuláková and D. Malyková (Institute of Archaeology of the Czech Academy of Sciences, Prague) between the years 2007 and 2016. Within the enclosed area, A. Danielisová uncovered an Early Únětice culture cemetery (BA1) in 2007. It contained multiple graves (33 skeletons in 13 graves), clearly earlier than the enclosure itself. Additional, isolated graves were spread over the site, along with some "irregular" burials in settlement pits. Ten individuals from two Early Únětice graves (nos. 63 and 76), two individuals from a tumulus (No. 690) and one individual from a settlement pit (No. 798) were analysed for aDNA. The cemetery, as well as the settlement, were analysed by M. Langová (*124*, *125*) and already partly published (*101*, *126*, *127*).

Grave 63. Grave pit with four skeletons. Two child skeletons in crouched position (skeletons 3 and 4), apparently related, were found in a niche in the western part of the grave. A coincidental superposition of two independent graves cannot be ruled out.

Skeleton 1. Skeleton (upper): right-sided crouched burial, head towards the south. Sex: anthropology – F?, aDNA – F. Age: adultus I (20–35). Grave goods: two vessels (which could also belong to skeleton 2) – a cup with fringe decoration and a small jug; two animal bones (scapulae). Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: MAMS-30775 (3677±21) 2137–1980 cal BC 2-sigma *(124)*. Pandora No.: BNL001. NM Prague Inv. No.: P7A 42041.

Skeleton 2. Skeleton (lower): crouched position, partly disrupted, head towards the south. Sex: anthropology – F?, aDNA – M. Age: adultus II (30-40). Grave goods: see skeleton 1. Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: not available *(124).* Pandora No.: BNL002. NM Prague Inv. No.: P7A 42042.

Skeleton 3. Skeleton: left-sided crouched burial, head towards the south, incompletely preserved. Sex: anthropology – child, aDNA – M. Age: infans (1.5). Grave goods: no finds. Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: MAMS-30776 (3661±24) 2135–1955 cal BC 2-sigma *(124).* Pandora No.: BNL003. NM Prague Inv. No.: P7A 42043.

Skeleton 4. Skeleton: crouched burial, head towards the south, incompletely preserved. Sex: anthropology – child, aDNA – M. Age: infans (7.5–9.5). Grave goods: no finds. Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: not available *(124).* Pandora No.: BNL004. NM Prague Inv. No.: P7A 42044.

Grave 76/204. Situated (in a superposition?) in (above?) the middle of a settlement pit (No. 76). Three people were gradually deposited here. Skeletons 2 and 3 were buried below skeleton 1. According to the find context, the original presence of a coffin or organic box is highly probable.

Skeleton 1. Skeleton (upper): right-sided crouched burial, head towards the south. Sex: anthropology – juvenis, aDNA – F. Age: juvenis (17±2). Grave goods: an undecorated cup. Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: MAMS-30777 (3738±23) 2206-2038 cal BC 2-sigma *(124).* Pandora No.: BNL005. NM Prague Inv. No.: P7A 42146/1.

Skeleton 2. Skeleton: below skeleton 1, in a non-anatomical position. Sex: anthropology – M, aDNA – M. Age: adultus II – maturus I (30–45). Grave goods: no finds. Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: not available *(124).* Pandora No.: BNL006. NM Prague Inv. No.: P7A 42146/2.

Skeleton 3. Skeleton: below skeleton 1, in a non-anatomical position. Sex: anthropology – M, aDNA – M. Age: maturus II (40–50). Grave goods: no grave goods. Archaeological dating: Early Únětice culture (BA1). Radiocarbon dating: not available *(124).* Pandora No.: BNL007. NM Prague Inv. No.: P7A 42146/3.

Grave 690. The settlement pit, secondarily enlarged and used for burying. It contained two skeletons in crouched position on their right side, heads towards the south. Skeleton 1 was situated above skeleton 2, separated by ca. 0.4 m of grave fill. Due to a circle of massive stones situated around the pit, the whole feature was originally reported as a tumulus.

Skeleton 1. Skeleton (upper): right-sided crouched burial, head towards the south. Sex: anthropology – child, aDNA – F. Age: infans (11–13). Grave goods: a simple bronze temple ring, the front right paw of a dog. Archaeological dating: Late Únětice culture (BA2). Radiocarbon dating: MAMS-30778 (3615±26) 2035–1896 cal BC 2-sigma; CRL-11260 (3716±83) 2432–1891 cal BC 2-sigma; CRL-11194 (3540±112) 2201–1614 cal BC 2-sigma *(101, 124, 126, 127).* Pandora No.: BNL008. NM Prague Inv. No.: P7A 42153/1.

Skeleton 2. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – M, aDNA – M. Age: adultus II – maturus I (35–50). Grave goods: deep bowl with a horizontal handle. Archaeological dating: Late Únětice culture (BA2). Radiocarbon dating: MAMS-30779 (3649±24) 2132–1944 cal BC 2-sigma. Radiocarbon date from macroremains – Poz-47314 (3640±35) 2135–1912 cal BC 2-sigma *(101, 124, 126, 127).* Pandora No.: BNL009. NM Prague Inv. No.: P7A 42153/2.

Grave 798. A skeleton in a non-standard position in the settlement pit. The right arm of the skeleton was cut off and the leg was disjointed. Sex: anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: No grave goods related to the skeleton, numerous potsherds in the fill of the pit. Archaeological dating: Late Únětice culture (BA2). Radiocarbon dating: MAMS-30780 (3505±25) 1897–1749 cal BC 2-sigma. Literature *(124, 126, 127).* Pandora No.: BNL010. NM Prague Inv. No.: P7A 42155.

4. Brandýsek (Brandýsek, Kladno district, central Bohemia, Czech Republic)

Published in (5)

The Eneolithic cemetery in the sandpit of Brandýsek was excavated by O. Kytlicová in 1955–1958. Before this excavation, many graves were destroyed by sand pitting, the known cemetery is, therefore, hardly complete. According to Kytlicová, about one half of the graves were preserved. In addition to a Neolithic settlement, Roman Iron Age cemetery and medieval graves, the excavation area of roughly 0.15 ha revealed at least two inhumation graves of the Funnel Beaker culture (one analysed by aDNA), 5–6 inhumation graves of the Corded Ware culture (two analysed) and 22 inhumation graves of the Bell Beaker culture (9 burials from 8 inhumation graves analysed). The Eneolithic cemetery was published in full *(128).* For a spatial analysis of the site see *(129)*.

Grave 3. Skeleton: heavily damaged by a bulldozer, the position and orientation could not be determined. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: juvenis (15–20). Grave goods: cup and fragments of two other cups. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4499 (3670±20 BP) 2136–1977 cal BC 2-sigma *(5, 128)*. Master ID and/or other aDNA signs: I7249, BRAN_3. NM Prague Inv. No.: P7A 31479.

Grave 4. Skeleton: right-sided crouched burial, disturbed, head towards the south. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: adultus (20–30). Grave goods: polypod bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not availabe *(5, 128)*. Master ID and/or other aDNA signs: I7250, BRAN_4. NM Prague Inv. No.: P7A 31480.

Grave 8. Skeleton: heavily disturbed, head towards the north. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (7–15). Grave goods: fragments of bowl. Archaeological dating: Bell Beaker culture, middle/late stage. Radiocarbon dating: not available *(5, 128, 130)*. Master ID and/or other aDNA signs: I7251, BRAN_8. NM Prague Inv. No.: P7A 31615.

Grave 12. Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: juvenis (15–18). Grave goods: no finds. Archaeological dating: Bell Beaker culture. Radiocarbon dating: not available *(5, 128, 130)*. Master ID and/or other aDNA signs: I7269, BRAN_12. NM Prague Inv. No.: P7A 31619.

Grave 19. Grave with two burials.

Skeleton 19A: right-sided crouched burial, head towards the south-west. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: see burial 19B. Archaeological dating: Bell Beaker culture, middle/late stage. Radiocarbon dating: not available *(5, 128)*. Master ID and/or other aDNA signs: I7270, BRAN_19A. NM Prague Inv. No.: P7A 31626A.

Skeleton 19B: left-sided crouched burial, head towards the north-east. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: decorated bell beaker, cup, bowl. Archaeological dating: Bell Beaker culture, middle/late stage. Radiocarbon dating: not available *(5, 128, 130)*. Master ID and/or other aDNA signs: I7271, BRAN_19B. NM Prague Inv. No.: P7A 31626B.

Grave 23. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (4–6). Grave goods: small beaker, cow pelvis. Archaeological dating: Funnel Beaker culture (Baalberge). Radiocarbon dating: PSUAMS-4239 (4715±20 BP) 3630–3377 cal BC 2-sigma *(5, 128, 130)*. Master ID and/or other aDNA signs: I7272, BRAN_23. NM Prague Inv. No.: P7A 31630.

Grave 26. Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: bowl, bone pendant. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 128, 130)*. Master ID and/or other aDNA signs: I7275, BRAN_26. NM Prague Inv. No.: P7A 31633.

Grave 34. Skeleton: heavily disturbed, the position and orientation of the skeleton could not be determined. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: infans (2–3). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 128, 130)*. Master ID and/or other aDNA signs: I7276, BRAN_34. NM Prague Inv. No.: P7A 31641.

Grave 71. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: jug, cup, bowl, small copper dagger, chipped industry – four arrowheads and two flakes. Archaeological dating: Bell Beaker culture, middle/late stage. Radiocarbon dating: PSUAMS-4349 (3890±20 BP) 2464–2299 cal BC 2-sigma *(5, 128, 130)*. Master ID and/or other aDNA signs: I7278, BRAN_71 NM Prague Inv. No.: P7A 31677.

Grave 74. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (5–6). Grave goods: beaker, amphora. Archaeological dating: Corded Ware culture, late stage. Radiocarbon dating: not available *(5, 128)*. Master ID and/or other aDNA signs: I7279, BRAN_74. NM Prague Inv. No.: P7A 32160.

Grave 78. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (3–4). Grave goods: beaker. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: not available *(5, 128)*. Master ID and/or other aDNA signs: I7280, BRAN_78. NM Prague Inv. No.: P7A 32163.

5. Březno u Loun (Březno u Loun, Louny district, NW Bohemia, Czech Republic)

Contact person: Miroslav Dobeš

The long-term (´systematic´) excavation of a multicultural site (evidence of residential and burial activities from the Neolithic up to the Early Middle Ages) in 1966, 1969–1970 and 1973–75. Among other finds, remains of two Proto-Eneolithic megadendric long barrows (I. Pleinerová) were also uncovered. Although their above-ground parts were not preserved, their area was delimited by peripheral trenches cut into the subsoil. The first of these (barrow 62) was ca. 24 m long and 3 m wide, with two Eneolithic graves located inside along its axis. Based on 14C dates, the grave in the front of the barrow (LXXIII) was primary, while the grave in the middle was added later (LXXV). The second barrow (No. 86) was ca. 144 m long and 4 m wide. Primary grave (CXV) was located in its front. Elongated grooves found in the subsoil inside the barrow, parallel to its longitudinal axis, were interpreted as plough marks. According to the stratigraphic contexts, they could chronologically correspond to the period of the barrow construction. Large numbers of additional graves (Corded Ware culture, Bell Beaker culture, Únětice culture) were found ´inside´ of both barrows, being also oriented along their longer axes, i.e. in the east-west direction. These later burials were apparently inserted into the mounds intentionally, when the mound bodies were still visible on the ground surface. Both barrows have been published in detail in *(100, 131)*.

Long barrow, feature no. 62, grave LXXIII. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – ?, anthropology – M, aDNA – M. Age: maturus II (50–60). Grave goods: small bowl/cup. Archaeological dating: Jordanów/Michelsberg culture. Radiocarbon dating: GrN-8803 (5090±45) 3974–3783 cal BC 2-sigma; MAMS-41371 (5078±25) 3956–3799 cal BC 2-sigma *(100, 131).* Pandora No.: BRZ001. NM Prague Inv. No.: P7A 35523.

Long barrow, feature no. 62, grave LXXV. Skeleton: left-sided crouched burial, head towards the west. Sex: archaeology – ?, anthropology – M?, aDNA – F. Age: maturus II (50–60). Grave goods: no finds. Archaeological dating: Funnel Beaker culture. Radiocarbon dating: MAMS-41372 (4768±26) 3638–3390 cal BC 2-sigma *(100, 131).* Pandora No.: BRZ002. NM Prague Inv. No.: P7A 35525.

Long barrow no. of feature 86, grave CXV. Skeleton: right-sided crouched burial, head towards the east. Sex: archaeology – ?, anthropology – F?, aDNA – no aDNA. Age: adultus I (20–30). Grave goods: small bowl. Archaeological dating: Jordanów/Michesberg culture. Radiocarbon dating: not available *(100, 131)*. Pandora No.: BRZ003. NM Prague Inv. No.: P7A 37013.

6. Čachovice (Čachovice, Chomutov district, NW Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský

The site was uncovered during a rescue excavation of Z. Smrž in front of an open-cast brown coal mine in 1980–1982. Next to the Neolithic settlement, remains of a Proto-Eneolithic or Early Eneolithic long barrow were found, as well as cemeteries of the Corded Ware and Bell Beaker cultures. The Corded Ware cemetery consisted of three clearly separated groups, with 59 graves and 60 inhumation burials altogether. In addition to pottery, the graves were furnished with lithic axes, axe-hammers and clubs, whetstones, chipped industry and even some bone and copper artefacts. According to the finds, the graves date to both the early and late periods of the Corded Ware culture. The Bell Beaker cemetery (21 inhumation graves) belonged to the late period of this culture; some of the graves clearly disturbed the Corded Ware grave pits. With the exception of the Neolithic settlement, all finds from this site were published in full (*132*). The cemetery was analysed for aDNA very selectively due to the poor preservation state of bones.

Grave 3. Skeleton: right-sided crouched burial, head towards the north-west. Sex: archaeology – M, anthropology – M?, aDNA – no aDNA. Age: adultus (20–40). Grave goods: beaker, amphora, stone club, stone axe, silicite axe, chipped industry – three flakes. Archaeological dating: Corded Ware culture, middle/late stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH001. NM Prague Inv. No.: P7A 38560.

Grave 7. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F, aDNA – no aDNA. Age: adultus I (20–30). Grave goods: amphora, jug, pot, single-handled cup, two-handled cup, two copper spiral temple rings, copper beads, chipped industry – one blade, whetstone. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH002. NM Prague Inv. No.: P7A 38563.

Grave 10. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – no aDNA. Age: maturus I (40–50). Grave goods: amphora, jug?,

stone battle axe, stone flat axe, chipped industry – two blades. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH003. NM Prague Inv. No.: P7A 38565.

Grave 11A. Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – F, anthropology – M, aDNA – no aDNA. Age: adultus I (20–30). Grave goods: amphora, jug, pot, two-handled cup, copper spiral temple ring, copper bead. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH004. NM Prague Inv. No.: P7A 38566.

Grave 12. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus I (20–30). Grave goods: amphora, jug/cup, stone battle axe, chipped industry – blade, bone awl, boar/pig tusk. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-41377 (3917±26) 2474–2306 cal BC 2-sigma *(132).* Pandora No.: CAH005. NM Prague Inv. No.: P7A 38568.

Grave 14. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M?, aDNA – no aDNA. Age: adultus (20–40). Grave goods: amphora, beaker, beaker with a handle, stone club, stone flat axe, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH006. NM Prague Inv. No.: P7A 38569.

Grave 24A. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus (20–40). Grave goods: two amphorae, jug, pot, chipped industry – blade, animal bones (drilled teeth?), whetstone. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH007. NM Prague Inv. No.: P7A 38576.

Grave 35. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – no aDNA. Age: adultus II – maturus I (30–50). Grave goods: amphora, beaker with a handle, stone battle axe, stone flat axe, chipped industry – blade and flake. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH008. NM Prague Inv. No.: P7A 38582.

Grave 46. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F?, aDNA – no aDNA. Age: adultus (20–40). Grave goods: three amphorae/jugs/pots, beaker, cylindrical beaker, chipped industry – two blades. Archaeological dating: Corded Ware culture, middle/late stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH009. NM Prague Inv. No.: P7A 38590.

Grave 47. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: amphora, jug, pot, single-handled cup, copper spiral temple ring. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-41378 (3853±27) 2457–2209 cal BC 2-sigma *(132).* Pandora No.: CAH010. NM Prague Inv. No.: P7A 38591.

Grave 92. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adult (over 30). Grave goods: amphora, two jugs, pot + more fragments of more vessels, blade from stone flat axe (?). Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(132).* Pandora No.: CAH011. NM Prague Inv. No.: P7A 38556.

7. Droužkovice (Droužkovice, Chomutov district, NW Bohemia, Czech Republic)

Contact person: Miroslav Dobeš

Rescue excavation above the seams of an underground brown coal mine (Z. Smrž) in 1982–1986. Three aceramic graves of the Corded Ware culture were found here, next to the multicultural prehistoric settlement remains (Funnel Beaker culture, Bell Beaker culture, Bronze Age, Early La Tène period). The graves contained either no finds or chipped industry only. Due to the state of bone preservation, only the following grave was analysed for the aDNA. The graves were published in full in *(133)*.

Grave 20/B2. Skeleton: right-sided crouched burial, head towards the west-north-west. Sex: archaeology – M, anthropology – ?, aDNA – M. Age: juvenis – adultus I (17–25). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-45783 (4147±23) 2872–2633 cal BC 2-sigma *(133).* Pandora No.: DRO001. NM Prague.

8. Holubice (Holubice, Prague-West district, central Bohemia, Czech Republic)

Contact person: Miroslav Dobeš, Josef Hložek, Erika Průchová

Rescue excavation on the construction of roadways and utility lines for a new residential zone in 2008 (J. Hložek). Within an area of roughly 0.1 ha, a multicultural site was uncovered, in which an inhumation grave of the Únětice culture, eight cremation graves and one inhumation burial from the Late to Final Bronze Ages, two iron furnaces and other pits from the Roman Iron Age and the inhumation graves of the Funnel Beaker and Baden cultures (described below) were found. The Baden culture inhumation grave is among the first of its kind in Bohemia. The graves were published in full in *(134)*.

Grave 21. Skeleton: right-sided crouched burial, head towards the south-east. Sex: archaeology – ?, anthropology – F ?, aDNA – F. Age: adultus II (30–40). Grave goods: beaker. Archaeological dating: Funnel Beaker culture, Baalberge/Siřem stage. Radiocarbon dating: CRL-15611 (4879±36) 3760–3539 cal BC 2-sigma *(134).* Pandora No.: HOL001. M Roztoky.

Grave 24. Grave with two burials.

Skeleton 1 (A): left-sided crouched burial, head towards the east-north-east. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: maturus I (40–50). Grave goods: two jugs, stone flat axe, drilled antler axe. Archaeological dating: Baden culture, classical stage. Radiocarbon dating: CRL-15612 (4486±36) 3347–3031 cal BC 2-sigma *(134).* Pandora No.: HOL004. M Roztoky.

Skeleton 2 (B): left-sided crouched burial, head towards the east-north-east. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: adultus II – maturus (30–55). Grave goods: see skeleton 1. Archaeological dating: Baden culture, classical stage. Radiocarbon dating: not available *(134).* Pandora No.: HOL002. M Roztoky.

9. Hostivice (Hostivice, Prague-West district, central Bohemia, Czech Republic)

Contact persons: David Daněček, Jana Klementová, Miluše Dobisíková

Rescue excavation by J. Klementová and D. Daněček (Museum Roztoky) in 2007-2008. The excavated area covers 10 ha, more than 1,300 settlement pit features and a similar number of post holes have been found. The site was occupied during the Linear and Stroked Pottery cultures, the Funnel Beaker and Řivnáč cultures, Hallstatt (Ha C-D1), Roman Iron Age and Early Middle Ages. More than 33 graves were also uncovered and burials were identified in sunken settlement features from the Funnel Beaker (Baalberge stage), Corded Ware, Bell Beaker and Knovíz cultures (B D – Ha A2), as well as from the La Tène period (Lt B1-C). A short report was published in *(135)*. Anthropological research was performed by M. Dobisíková (National Museum). Four skeletons from eight graves belonging to the Bell Beaker culture (features 688, 689, 690 and 691) were sampled for aDNA.

Grave 17 (Feature 688). Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: adultus II – maturus I (35–50). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30798 (3837±24) 2455–2202 cal BC 2-sigma. (Not published). Pandora No.: HOP001A, B. NM Prague Inv. No.: P7A 18906.

Grave 19 (Feature 689). Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: adultus II – maturus I (35–50). Grave goods: three cups, bowl, pot. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30799 (3795±25) 2294–2141 cal BC 2-sigma. (Not published). Pandora No.: HOP002A, B. NM Prague Inv. No.: P7A 18907.

Grave 20 (Feature 690). Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus II (50–60). Grave goods: no finds. Archaeological dating: Bell Beaker culture, late stage (?). Radiocarbon dating: not available. (Not published). Pandora No.: HOP003A, B, C. NM Prague Inv. No.: P7A 18908.

Grave 22 (Feature 691). Skeleton: left-sided (!) crouched burial, head towards the south. Sex: archaeology – atypical, anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: no finds. Archaeological dating: Bell Beaker culture, late stage (?). Radiocarbon dating: MAMS-38921 (3826±27) 2450–2150 cal BC 2-sigma. (Not published). Pandora No.: HOP004A, B, C. NM Prague Inv. No.: P7A 18909.

10. Chleby (Nymburk district, central Bohemia, Czech Republic)

Contact person: Petr Krištuf, Ondřej Švejcar, Erika Průchová, Michal Ernée

In 2016, a grave with a single burial (no. 2035) and a collective inhumation grave (no. 2036) from the early stage of the Únětice culture were uncovered during the excavation by the University of West Bohemia in Pilsen. Collective grave no. 2036 contained skeletal remains of at least 15 more or less complete individuals.

Grave/Skeleton 2035. Skeleton: partly disturbed, Sex: anthropology – child, aDNA – M. Age: infans (3–5). Grave goods: two vessels. Radiocarbon dating: not available. Pandora No.: CHL008.A, B.

Únětice collective grave, feature no. 2036

Skeletons: Collective grave no. 2036 contained more or less complete skeletal remnants of at least 15 individuals. Three skeletons were found in an anatomically correct position (2038, 2039 and 2077), three skeletons were partly disturbed and without skulls (2079-1, 2079-2 and 2080). There were also four separately deposited skulls (2041, 2075, 2076 and 2078) and a disarticulated deposition of human bones from other skeletons mixed with animal bones (2040). Grave goods: Five vessels, animal bones. Archaeological dating: stage 2–3 (early and middle stages) of the Únětice culture after Moucha *(112)*.

Skeleton 2038. Skeleton: anatomically correct position, almost completely preserved. Sex: anthropology – M, aDNA – M. Age: adultus I (22–24). Radiocarbon dating: MAMS-40617 (3623±28) 2116–1900 cal BC 2-sigma. Pandora No.: CHL003.

Skeleton 2039. Skeleton: anatomically correct position, almost completely preserved, Sex: anthropology – M, aDNA – M. Age: adultus I (20–30 /22–24/). Radiocarbon dating: not available. Pandora No.: CHL002.

Skeleton (skull) 2040/1. Skeleton: disarticulated, about 40% preserved, Sex: anthropology – M, aDNA – F. Age: adultus I (20–30). Radiocarbon dating: not available. Pandora No.: CHL001.

Skeleton (skull) 2041. Skeleton: only skull without mandibula, Sex: anthropology – F, aDNA – F. Age: maturus I (40–50). Radiocarbon dating: not available. Pandora No.: CHL007.

Skeleton (skull) 2075. Skeleton: only skull without mandibula, Sex: anthropology – M, aDNA – no aDNA. Age: adultus I–II (24–35). Radiocarbon dating: not available. Pandora No.: CHL004.

Skeleton (skull) 2076. Skeleton: only skull without mandibula, Sex: anthropology – F, aDNA – M. Age: senilis (over 55). Radiocarbon dating: not available. Pandora No.: CHL005.

Skeleton (skull) 2078. Skeleton: isolated skull, Sex: anthropology – child, aDNA – F. Age: infans (5–9). Radiocarbon dating: MAMS-40618 (3591±28) 2024–1887 cal BC 2-sigma. Pandora No.: CHL006.

11. Kněževes 1 (Prague-West district, central Bohemia, Czech Republic)

Published in (4, 5)

Rescue excavation before the construction of a family home (O. Kytlicová, AI Prague) in 1953–1954. Within an area of ca. 100 m^2, 12 inhumation and two bi-ritual Bell Beaker graves from the late stage of this culture were uncovered. The graves were published in full in *(136)* and *(137)*. Two of them were sampled for aDNA (*4*, *5*) and Sr *(138).*

Grave 14. Skeleton: left sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans. Grave goods: bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: Poz-84460 (3740±35 BP) 2279–2033 cal BC 2-sigma *(4, 5, 136, 137).* Referred to in *(5)* as I5024. Master ID and/or other aDNA signs: I4145, RISE566, F0521. NM Prague Inv. No.: P7A 31168.

Grave 8. Skeleton: left sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – F. Age: juvenis (17–19). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(4, 5, 136, 137).*

Referred to in *(5)* as I5025. Master ID and/or other aDNA signs: I4136, RISE567, F0523 NM. Prague Inv. No.: P7A 30766.

12. Kněževes 2 (Prague-West district, central Bohemia, Czech Republic)

Contact persons: Lubor Smejtek, Michal Ernée

Rescue excavation by L. Smejtek (Archaia Prague) in 1998. Within an area of more than 10 ha, a total of 2,939 sunken settlement features were excavated, dating mostly (87.5%) to the Late and Final Bronze Ages. Completely published by L. Smejtek (*139*, *140*). A total of 23 inhumation graves and some sunken settlement features from the early stage of the Early Bronze Age Únětice culture (*140*, *141*) were recorded, too. A total of seven skeletons from five graves (features 2225, 2229, 2234, 2351 and 2767) were sampled for aDNA.

Grave 2225. Grave pit with two skeletons: A (above) and B (below, maturus, 40–60). Fragments of a willow-leaf-type earring made of bronze/copper were found near the skull of individual A. Six vessels belonged probably to the skeleton B. Archaeological dating: early stage of the Únětice culture (Early Únětice stage after the periodisation of V. Moucha).

Skeleton A. Skeleton: preserved only some skull bones, some teeth and bones of one hand; original position of the skeleton unknown, head towards the south-south-east. Sex: anthropology – ?, aDNA – F. Age: maturus I (40–50). Grave goods: fragments of a copper/bronze earring of the willow-leaf-type. Radiocarbon dating: MAMS-30771 (3705±24) 2195–2028 cal BC 2-sigma *(140, 141).* Pandora No.: KNE002. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

Grave 2229. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F?, aDNA – F. Age: juvenis (13–20). Grave goods: two vessels and one pin made of bone. Archaeological dating: early stage of the Únětice culture (Early Únětice stage after periodisation of V. Moucha). Radiocarbon dating: MAMS-30772 (3648±19) 2126–1949 cal BC 2-sigma *(140, 141).* Pandora No.: KNE003. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

Grave 2234. Archaeological dating: early stage of the Únětice culture (Early Únětice stage after V. Moucha).

Skeleton A. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: maturus I (40–50). Grave goods: bronze earring, probably two vessels (1–2). Radiocarbon dating: MAMS-30773 (3704±24) 2195–2027 cal BC 2-sigma *(140, 141).* Pandora No.: KNE004. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

Skeleton B. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – ?, aDNA – F. Age: adultus I (20–30). Grave goods: three vessels (3–5). Radiocarbon dating: MAMS-38478 (3730±24) 2201–2037 cal BC 2-sigma *(140, 141).* Pandora No.: KNE005. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

Skeleton C. Skeleton: preserved only small parts of skull and some teeth, perhaps manipulated. Sex: anthropology – ?, aDNA – F. Age: adultus II (30–40). Grave goods: no grave goods. Radiocarbon dating: not available *(140, 141).* Pandora No.: KNE006. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

Grave 2351. Skeleton: right-sided crouched burial, head towards the south-east. Sex: anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: two vessels, including typical jug of the Proto-Únětice culture (vessel 2). Archaeological dating: earliest stage of the Únětice culture (Proto-Únětice stage after periodisation of V. Moucha). Radiocarbon dating: Bln-5436 (3679±34) 2193–1957 cal BC 2-sigma *(140-142).* Pandora No.: KNE001. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

Grave 2767. Skeleton: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: three vessels (two dishes). Archaeological dating: early stage of the Únětice culture (Middle Únětice stage after periodisation of V. Moucha). Radiocarbon dating: MAMS-30774 (3651±20) 2129–1950 cal BC 2-sigma *(140, 141).* Pandora No.: KNE007. ÚAPPSČ – Institute of Archaeological Heritage of Central Bohemia.

13–16. Kolín, road bypass (Kolín, Kolín district, central Bohemia, Czech Republic)

Rescue excavation preceding the construction of the road bypass around the town in 2008-2010, directed by R. Šumberová and D. Malyková (Institute of Archaeology, Prague). The length of the excavated strip of land was about 8 km and an area of ca. 40 ha was completely uncovered. Excavated 7,000 features belong to most of the archaeological periods between the beginning of the Neolithic up to the Early Middle Ages. The excavation was divided into ten sections (Kolín I-X), treated as separate fieldwork events with their own context numbers. Finds have been published selectively so far, for information see *(143, 144)*.

13. Kolín, road bypass, site I

Excavation by R. Šumberová in 2008–2010. Remains of a multicultural site with many settlement components (Neolithic, Funnel Beaker, Baden, Řivnáč, Globular Amphora, Únětice cultures, Middle Bronze Age, Hallstatt period) and burial areas (Únětice and Corded Ware cultures, Early Middle Ages) were uncovered.

13.1. Kolín, road bypass, site I-3

Published in (62)

The grave under study was discovered in the fill of the ditch of the Late Neolithic rondel 2, at the depth of 120 cm below the surrounding subsoil surface level. The analysis of the field situation indicates that the burial was laid down into an partially filled rondel ditch, still well visible from the landscape surface even several centuries after its abandonment.

Grave 265. Skeleton: right-sided, subtly crouched burial, partially on the back, head towards the south. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: adultus I (20–25). Grave goods: beaker, bowl. Archaeological dating: Stroked Pottery culture. Radiocarbon dating: UGAMS-9614 (5710±25 BP) 4650–4460 cal BC 2-sigma *(62, 145)*. Master ID and/or other aDNA signs: kol002. Institute of Archaeology in Prague, Kutná Hora Branch.

13.2. Kolín, road bypass, site I-7a

Published in (62)

The grave described below was uncovered in a shallow ´building´ pit alongside a Linear Pottery culture house. Most probably it represents a secondary intervention into an earlier feature.

Grave 5160. Skeleton: semi-flexed left-sided position, oriented north-south with the face turned towards the east. The burial pit was so small and constricted that the woman's trunk – supported by the sloping pit wall – gave the impression of an almost sitting skeleton. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: adultus I (20–25). Grave goods (?): small sharp point made of bone. Archaeological dating: Stroked Pottery culture. Radiocarbon dating: UGAMS-9615 (5950±25 BP) 4910–4740 cal BC 2-sigma *(62, 144)*. Master ID and/or other aDNA signs: kol006. Institute of Archaeology in Prague, Kutná Hora Branch.

13.3. Kolín, road bypass, site I-7b

Contact persons: Radka Šumberová, Hana Brzobohatá, Miroslav Dobeš, Michal Ernée

The aDNA analyses were performed on two inhumation burials in the fill of the a semi-sunken Řivnáč culture hut (feature 3790), one inhumation burial of the Corded Ware culture (feature 3013), three burials from the Únětice culture settlement pit (feature 3037, group A; two individuals from other settlement pits were not analysed) and, selectively, skeletons from the Únětice culture cemetery (a total of 67 graves with 75 individuals, from which six graves with seven skeletons have been selected – see below for details, group B). With the exception of the Řivnáč culture feature 3790 (*146*, *147*), these finds have not been published yet (for preliminary information see *(143, 144)*).

Feature No. 3790. Two irregular inhumation burials in a semi-sunken hut.

Skeleton 1. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: adultus II – maturus I (30–50). With the exception of fragments of a jug (?), no finds can be directly connected with the skeleton (only settlement discard from the fill of the feature). Archaeological dating: Řivnáč culture. Radiocarbon dating: MAMS-30781 (4216±24) 2898–2700 cal BC 2-sigma *(146, 147).* Pandora No.: KO1001A, B. Institute of Archaeology in Prague, Kutná Hora Branch.

Skeleton 2. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (2–3). No connectable goods, only settlement discard from the fill of the feature. Archaeological dating: Řivnáč culture. Radiocarbon dating: not available *(146, 147).* Pandora No.: KO1016. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 3013. Skeleton: right-sided crouched burial, head towards the west-south-west. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: adultus – maturus I (25–50). Grave goods: flat stone axe. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-38481 (4055±29) 2835–2485 cal BC 2-sigma. (Not published). Pandora No.: KO1002A. Institute of Archaeology in Prague, Kutná Hora Branch.

Group A

Settlement pit, feature No. 3037. The settlement pit (round, diameter 235 cm, depth 30 cm) has been used for burying of three individuals, one adult male and two children. The skeletons were partly covered with fragments of vessels and grinding stones under a layer with a large amount of potsherds, stones and daub. One vessel was found under the skeletons in the middle of the pit.

Skeleton I: buried in prone position. Sex: anthropology – child, aDNA – F. Age: infans (5–7). Grave goods: no personal grave goods. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UGAMS-9623 (3570±25) 2017–1784 cal BC 2-sigma. Pandora No.: KO1006. Institute of Archaeology in Prague, Kutná Hora Branch.

Skeleton II: buried in supine position. Sex: anthropology – child, aDNA – M. Age: infans (8–10). Grave goods: no personal grave goods. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30785 (3460±25) 1879–1694 cal BC 2-sigma. Pandora No.: KO1007. Institute of Archaeology in Prague, Kutná Hora Branch.

Skeleton III: right-sided crouched burial, head towards the south. Sex: anthropology – M, aDNA – M. Age: adultus (20–40). Grave goods: no personal grave goods. Archaeological dating: Classic Únětice culture. Radiocarbon dating: not available. Pandora No.: KO1008. Institute of Archaeology in Prague, Kutná Hora Branch.

Group B

Grave 3540. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: two small vessels, one bronze earring, scapula. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UGAMS-9621 (3570±25) 2017–1784 cal BC 2-sigma; MAMS-30786 (3589±24) 2021–1888 cal BC 2-sigma. Pandora No.: KO1009. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 3690. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: adultus I (20–25). Grave goods: one small vessel, bronze pin of the Únětice type, two bronze beads, amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30787 (3599±24) 2022–1895 cal BC 2-sigma; CRL-19323 (3556±26) 2009–1777 cal BC 2-sigma. Pandora No.: KO1010. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 3829. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – no aDNA. Age: juvenis (16–18). Grave goods: bronze arm-ring, bone tools, and amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30788 (3503±25) 1894–1749 cal BC 2-sigma. Pandora No.: KO1012. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 4316. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: three vessels. Archaeological dating: early stage of the Únětice culture (Middle or Pre-Classic Únětice stage after V. Moucha). Radiocarbon dating: UGAMS-9622 (3660±25) 2135–1952 cal BC 2-sigma. Pandora No.: KO1011. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 4332

Skeleton I: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: three vessels, bronze pin of the Cypriot type, three

bronze earrings, three bronze tubular spirals, hook with bone discs (float?), animal bone (scapula). Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30789 (3605±24) 2025–1899 cal BC 2-sigma (human bone); CRL-19325 (3528±28) 1939–1766 cal BC 2-sigma (animal scapula). Pandora No.: KO1013. Institute of Archaeology in Prague, Kutná Hora Branch.

Skeleton II: child's bones by the woman's leg. Sex: anthropology – child, aDNA – M. Age: infans (8–11). Grave goods: Archaeological dating: Classic Únětice culture. Radiocarbon dating: not available. Pandora No.: KO1014. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 4347. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: adultus II – maturus I (30–50). Grave goods: two small vessels, bronze pin, bronze earring. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30790 (3509±24) 1900–1752 cal BC 2-sigma. Pandora No.: KO1015. Institute of Archaeology in Prague, Kutná Hora Branch.

14. Kolín, road bypass, site II

Contact person: Radka Šumberová, Hana Brzobohatá

Excavation of R. Šumberová in 2008. A multicultural site, settlement components prevailing (Neolithic, Funnel Beaker, Řivnáč/Bošáca and Únětice cultures, Middle Bronze Age up to the La Tène period); a small number of graves (four Bell Beaker culture graves, one La Tène period grave). Three Bell Beaker (late stage) inhumation graves described below have been chosen for the aDNA analysis. All three were published in full *(148)*.

Grave 4071. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: cup, bowl, chipped industry – two arrowheads and dagger (?). Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30782 (3783±25) 2287–2140 cal BC 2-sigma *(148).* Pandora No.: KO1003A, B, C. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 4073. Skeleton: left-sided crouched burial, head towards the north-east-north. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: juvenis – adultus I (18–22). Grave goods: cup, handled pot, bone artefact – finger ring. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30783 (3787±24) 2288–2141 cal BC 2-sigma *(148).* Pandora No.: KO1004A, B. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 4104. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: two cups, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30784 (3892±24) 2464–2299 cal BC 2-sigma *(148).* Pandora No.: KO1005A, Institute of Archaeology in Prague, Kutná Hora Branch.

15. Kolín, road bypass, Site VI – Polepy

Contact persons: Radka Šumberová, Hana Brzobohatá

Rescue excavation of R. Šumberová due to the construction of the Kolín road bypass in 2009, section VI (cadastre area Polepy). A multicultural site with prevailing settlement components (Řivnáč, Bell Beaker and Únětice cultures, Hallstatt period) and a small group of graves (eight Bell Beaker graves). Three Bell Beaker culture (late stage) inhumation graves described below were chosen for aDNA analysis. Publication of the finds is being prepared.

Grave 4986. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (6–10). Grave goods: handled pot. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available. (Not published). Pandora No.: KOP001A, B. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 4989. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (8–12). Grave goods: cup, bowl, animal bones. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available. (Not published). Pandora No.: KOP002A, B, C, D. Institute of Archaeology in Prague, Kutná Hora Branch.

Grave 5216. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – ?, aDNA – M. Age: juvenis (14–18). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30791 (3907±24) 2470–2306 cal BC 2-sigma. (Not published). Pandora No.: KOP003A, B. Institute of Archaeology in Prague, Kutná Hora Branch.

16. Kolín, road bypass, Site VII

Contact person: Drahomíra Malyková, Hana Brzobohatá

During the rescue excavation of the road bypass in 2009 (D. Malyková), a small cemetery of the early Únětice culture with nine graves was documented. Three skeletons were sampled for aDNA.

Grave 1409. Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – ?, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: one vessel, bone awl, chipped industry. Archaeological dating: early Únětice culture. Radiocarbon dating: MAMS-30792 (3655±24) 2133–1949 cal BC 2-sigma. Pandora No.: KO7001. Institute of Archaeology in Prague.

Grave 1412. Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – F?, aDNA – F. Age: adultus II – maturus II (30–60). Grave goods: two vessels. Archaeological dating: early Únětice culture. Radiocarbon dating: not available. Pandora No.: KO7002. Institute of Archaeology in Prague.

Grave 1418. Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: two vessels. Archaeological dating: early Únětice culture. Radiocarbon dating: MAMS-30793 (3761±24) 2281–2050 cal BC 2-sigma. Pandora No.: KO7003. Institute of Archaeology in Prague.

17. Kolín-Šťáralka (Kolín, Kolín district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Miloš Vávra

Rescue excavation due to the construction of an industrial hall in 2008 (M. Vávra, IAHCB). On an area of 0,15 ha, a settlement site from the Neolithic and Early Bronze Age was uncovered, burial activities being represented by 14 inhumation graves from the Baalberge stage of the Funnel Beaker culture and one Corded Ware grave. Samples for aDNA analysis were taken from Funnel Beaker culture skeletons, in cases the preservation state allowed it (a total of 7 graves). Roughly 40% of the graves were furnished with pottery, while the others contained no finds: this means that their cultural affiliation can be deduced only from the form of the burial ritual. Given the condition of the bones, the cemetery was analysed only selectively; the results were published in full in *(149, 150).*

Grave 56/H1. Skeleton: left-sided crouched burial, head towards the north-west. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: juvenis – adultus I (18–24). Grave goods: fragments of one vessel. Archaeological dating: Funnel Beaker culture, Baalberge stage. Radiocarbon dating: not available *(149, 150).* Pandora No.: KOB001. Institute of Archaeological Heritage of Central Bohemia.

Grave 57/H2. Skeleton: left-sided crouched burial, head towards the west-north-west. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: maturus (45–55). Grave goods: beaker, jug, bowl, another vessel. Archaeological dating: Funnel Beaker culture, Baalberge stage. Radiocarbon dating: MAMS-45784 (4905±25) 3747–3642 cal BC 2-sigma *(149, 150).* Pandora No.: KOB002. Institute of Archaeological Heritage of Central Bohemia.

Grave 66/H5. Skeleton: left-sided crouched burial, head towards the west-north-west. Sex: archaeology – ?, anthropology – F, aDNA – M. Age: juvenis (14–16). Grave goods: no finds. Archaeological dating: Funnel Beaker culture, Baalberge stage. Radiocarbon dating: not available *(149, 150).* Pandora No.: KOB003. Institute of Archaeological Heritage of Central Bohemia.

Grave 68/H6. Skeleton: left-sided crouched burial, head towards the north-west. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: juvenis (14–18). Grave goods: fragments of jug, fragments other vessel. Archaeological dating: Funnel Beaker culture, Baalberge stage. Radiocarbon dating: not available *(149, 150).* Pandora No.: KOB004. Institute of Archaeological Heritage of Central Bohemia.

Grave 72/H10. Skeleton: left-sided crouched burial, head towards the west-north-west. Sex: archaeology – ?, anthropology – M, aDNA – F. Age: juvenis (14–16). Grave goods: no finds. Archaeological dating: Funnel Beaker culture, Baalberge stage. Radiocarbon dating: MAMS-45785 (4986±24) 3906–3701 cal BC 2-sigma *(149, 150).* Pandora No.: KOB005. Institute of Archaeological Heritage of Central Bohemia.

Grave 75/H13. Skeleton: left-sided crouched burial, head towards the west-north-west. Sex: archaeology – ?, anthropology – ?, aDNA – no aDNA. Age: maturus (45–55). Grave goods: fragments of one vessel. Archaeological dating: Funnel Beaker culture, Baalberge stage. Radiocarbon dating: not available *(149, 150).* Pandora No.: KOB006. Institute of Archaeological Heritage of Central Bohemia.

Grave 77/H14. Skeleton: left-sided crouched burial, head towards the north-west. Sex: archaeology – ?, anthropology – M, aDNA – M. Age: maturus (45–55). Grave goods: jug, two bowls, fragments of another vessel. Archaeological dating: Funnel Beaker culture, Baalberge

stage. Radiocarbon dating: MAMS-45786 (4908±25) 3756–3643 cal BC 2-sigma *(149, 150).* Pandora No.: KOB007. Institute of Archaeological Heritage of Central Bohemia.

18. Konobrže (Konobrže, Most district, NW Bohemia, Czech Republic)

Contact person: Miroslav Dobeš

Rescue excavation in the forefront of an open-cast coal mine (P. Čech, M. Dobeš, D. Koutecký) in 1991–1994. The excavation area extended to ca. 1 ha. Settlement finds belonged to the Řivnáč, Knovíz and Hallstatt cultures/periods, burial monuments were represented by an Únětice culture cemetery and 11 Corded Ware graves (early and later periods). In addition to pottery, the graves were furnished with chipped industry and bone and copper artefacts, including an antler belt clasp (see below). The Corded Ware culture cemetery was published in full in *(151, 152)*; the analysis for the aDNA was selective.

Grave 10A/91. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: adultus (20–40). Grave goods: beaker, chipped industry – blade. Archaeological dating: Corded Ware culture, early stage. Radiocarbon dating: MAMS-45787 (4147±24) 2872–2632 cal BC 2-sigma *(151).* Pandora No.: KON001. NM Prague.

Grave 26/91. Skeleton: left-sided crouched burial, head towards the east. Stratigraphically above grave 26A/91. Sex: archaeology – F, anthropology – F?, aDNA – no aDNA. Age: adultus II (30–40). Grave goods: amphora, pot, jug, cup, copper spiral temple ring. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(151).* Pandora No.: KON002. NM Prague.

Grave 26A/91. Skeleton: right-sided crouched burial, head towards the west. Stratigraphically beneath grave 26/91. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (around 2). Grave goods: drilled animal tooth, chipped industry – flake. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(151).* Pandora No.: KON003. NM Prague.

Grave 31/91. Skeleton: left-sided crouched burial, head towards the south-east. In the grave together with the skeleton of a newborn. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: amphora, jar, 370 animal teeth and their imitations, 43 bone beads, about one thousand shell beads, bone awl, animal bones (pig), chipped industry – blade. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-45788 (3947±24) 2564–2347 cal BC 2-sigma *(151).* Pandora No.: KON004. NM Prague.

Grave 26/94. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: two bone belt clasps, chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-45789 (4130±24) 2868–2586 cal BC 2-sigma *(152).* Pandora No.: KON005. NM Prague Inv. No.: P7A 39467.

19. Lochenice (Lochenice, Hradec Králové district, east Bohemia, Czech Republic)

Published in (5)

The site was excavated in two phases: in 1953–1954 (J. Tomský, Museum Hradec Králové) and in 1976–1983 (J. Zeman, Institute of Archaeology, Prague, in cooperation with M. Buchvaldek and J. Sláma, Charles University). A Neolithic rondel and graves from the Lusatian Urnfield culture, Migration Period and Middle Ages have been discovered, as well as a bi-ritual Bell Beaker culture cemetery with 23 graves *(153)*. Human remains from only one grave (described below) have been subjected to aDNA analysis to date.

Grave 1/82 (Buchv 20). Skeleton: heavily disturbed, grave pit was imperceptible. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (3–4). Grave goods: undecorated beaker (?), two cups, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 153).* Master ID and/or other aDNA signs: I5666, F0519. NM Prague Inv. No.: P7A 38408.

20. Lovosice (Litoměřice district, NW Bohemia, Czech Republic)

Published in (5)

Rescue excavation during the construction of the AOYAMA factory in the Lovosice industrial zone (M. Půlpán and V. Sušická, Institute of Archaeological Heritage Care, Most) in 2002. Besides a Řivnáč culture settlement, seven Corded Ware graves, 14 Iron Age graves and one (described below) Bell Beaker grave were investigated over an area of ca. 7 ha (unpublished to date).

Grave 4/2002. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: maturus I (40–50). Grave goods: decorated handled beaker. Archaeological dating: Bell Beaker culture, middle stage. Radiocarbon dating: not available *(5)*. Master ID and/or other aDNA signs: I6476, RISE736. Institute of Archaeological Heritage of North-West Bohemia.

21. Makotřasy (Kladno district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský.

The site was discovered and largely excavated in 1961, due to the construction of the highway to Slaný, small trenching followed in 1973–1991 (E. Pleslová-Štiková, Institute of Archaeology, Prague). Excavations and geophysical surveys identified a 9-ha square enclosure delimited by ditch II. This square enclosure was interpreted as a cult feature and dated to the Sířem stage of the Funnel Beaker culture. Documented inside and outside the enclosure were more than 150 settlement pits that were to have been dug shortly after the end of the enclosure's primary function (the settlement features again belong to the Sířem stage of the Funnel Beaker culture). The remains of ca. 50 human skeletons were found in ditch II and settlement features, twenty of which were subjected to aDNA analysis (listed below). Besides the dominant Funnel Beaker culture finds, occupation was also identified from the periods of the Jordanów, Řivnáč, Únětice (pit 143: Bln-3335, 3560±60 BP, 2119–1701 cal BC 2-sigma), Knovíz and La Tène cultures. Only the rescue excavation from 1961 (*154*) has been published in detail, while the others have been mentioned thus far only in the context of comprehensive articles (for the latest review, see *(55).*) *P. Velemínský* anthropologically evaluated the analysed skeletons using existing methods (for older assessments, see *(155)*).

Settlement pit 1/61 (burial 2, “east”). Skeleton: right-sided crouched burial, head towards the north-west. A second crouched burial was found in the pit (not analysed, inv. no. P7A 34203). Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: maturus – senilis (over 40). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: not available *(154, 155).* Master ID and/or other aDNA signs: I14175. NM Prague, Inv. No.: P7A 34204.

Settlement pit 20/61. Skeleton: right-sided crouched burial, head towards the north-west. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: adultus (25–35). Grave goods: probably a bowl behind the skull, the rest only settlement discard from the fill of feature. Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: PSUAMS-4231 (4990±25) 3914–3702 cal BC 2-sigma *(154, 155).* Master ID and/or other aDNA signs: I7186, MKTY_20. NM Prague, Inv. No.: P7A 34183.

Settlement pit 28/61. Skeletons: on the bottom of the feature, the remains of two disturbed (?) children’s burials, one of which was analysed (burial 2, skull on the left temple facing possibly towards the east). Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (4–6). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: not available *(154, 155).* Master ID and/or other aDNA signs: I14174. NM Prague, Inv. No.: P7A 34185.

Settlement pit 35/61. Skeleton: skull without mandible, on the right temple facing north. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: infans – juvenis (12–16). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: not available *(154, 155).* Master ID and/or other aDNA signs: I14170. NM Prague, Inv. No.: P7A 34187.

Settlement pit 51/61. Skeleton: right-sided crouched burial, head towards the north. Human bones of an additional two individuals found in the fill of the feature. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (6–8). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Jordanów (?) culture. Radiocarbon dating (data from the animal bones acquired from the fill of the same feature are not consistent with the radiocarbon date from the human bones): PSUAMS-4404 (5260±20) 4228–3988 cal BC 2-sigma (human bones); GrN-6928 (4550±110) 3623–2926 cal BC 2-sigma (0–20 cm, animal bones); GrN-6929 (4715±60) 3635–3371 cal BC 2-sigma (20–70 cm /bottom/, animal bones) *(154, 155).* Master ID and/or other aDNA signs: I7187, MKTY_51. NM Prague, Inv. No.: P7A 34191.

Settlement pit 59/61. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (3–5). Grave goods: probably a small beaker in front of the skull, further only settlement discard from the fill of feature. Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: not available *(154, 155).* Master ID and/or other aDNA signs: I14167. NM Prague, Inv. No.: P7A 34192.

Settlement pit 86b/61 (north). Skeleton: only skull without mandible. Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: adultus II – maturus (over 30). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: PSUAMS-4232 (4880±20) 3696–3641 cal BC 2-sigma *(154, 155).* Master ID and/or other aDNA signs: I7188, MKTY_86. NM Prague, Inv. No.: P7A 34194.

Settlement pit 96/61. The remains of four skeletons were found in the settlement pit. Burial No. 3 after (*154*) (inv. no. P7A 34195) was not analysed.

Skeleton 1 (burial 1): right-sided crouched burial, head towards the north. Found at the east end of the feature. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: juvenis (15–20). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: PSUAMS-3879 (4840±25) 3694–3535 cal BC 2-sigma *(154, 155).* Master ID and/or other aDNA signs: I7192, MKTY_96/3. NM Prague, Inv. No.: P7A 34197.

Skeleton 2-1 (burial 2): right-sided crouched burial, head towards the south-west. Found at the north-west end of the feature. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (6–8). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: PSUAMS-4233 (4765±20) 3636–3521 cal BC 2-sigma *(154, 155).* Master ID and/or other aDNA signs: I7189, MKTY_96/1. NM Prague, Inv. No.: P7A 34196.

Skeleton 2-2 (in *(154)* without number): disturbed burial at the west end of the feature. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (2–3). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: PSUAMS-3997 (4945±25) 3776–3657 cal BC 2-sigma *(154, 155).* Master ID and/or other aDNA signs: I7191, MKTY_96/2. NM Prague, Inv. No.: P7A 34196.

Settlement pit 100/61 (burial "e"). Skeleton: right-sided crouched burial, head towards the south-west. The remains of a disturbed burial were also found in the settlement pit (inv. no. P7A 34199). Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: infans – juvenis (12–15). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: not available *(154, 155).* Master ID and/or other aDNA signs: I14176. NM Prague, Inv. No.: P7A 34200.

Settlement pit 115/61. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: adultus I (20–25). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: not available *(154, 155).* Master ID and/or other aDNA signs: I14168. NM Prague, Inv. No.: P7A 34201.

Settlement pit 120/61. Skeleton: left-sided, mildly-crouched burial, with trunk turned on stomach, head towards the west. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: maturus – senilis (40+). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Sířem stage. Radiocarbon dating: PSUAMS-4234 (4890±25) 3706–3641 cal BC 2-sigma *(154, 155).* Master ID and/or other aDNA signs: I7193, MKTY_120. NM Prague, Inv. No.: P7A 34202.

Grave 124/75. Skeleton: right-sided crouched burial, head towards the south. Based on stratigraphy, probably Funnel Beaker culture. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (11–13). Without directly related goods. Archaeological dating: Funnel Beaker culture, Sířem stage? Radiocarbon dating: not available. (Not published, find report 3341/77). Master ID and/or other aDNA signs: I14171. NM Prague, Inv. No.: P7A 37239.

Test pit 19, feature 125/79. Skeleton: right-sided crouched burial, head towards the west. In the fill of ditch II, at a depth of 70–85 cm. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (8–10). Without directly related goods (only settlement discard from the fill of the ditch). Archaeological dating: Funnel Beaker culture, Siřem stage. Radiocarbon dating: not available. (Not published, find report 5312/81). Master ID and/or other aDNA signs: I14169. NM Prague, Inv. No.: P7A 38053.

Settlement pit 127/80. Skeleton: right-sided crouched burial covered with daub and stones, head towards the north-west. Found beneath the level of a feature interpreted as a furnace. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (2–4). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Funnel Beaker culture, Siřem stage. Radiocarbon dating: not available. (Not published, find report 5051/82). Master ID and/or other aDNA signs: I14172. NM Prague, Inv. No.: P7A 38218.

Test pit 27/81. Remains of at least seven individuals found on the east side of ditch II, at one of the entrances to the square enclosure. The skeletal remains rested at least partially in anatomical contexts on the bottom of the ditch, mostly in irreverential positions. A total of four burials were subjected to aDNA analysis. As such, the find situation supports a deposition of all individuals at the same time, most probably during a single burial event. The burials were not accompanied by goods, only settlement discard from the fill of the ditch.

Skeleton 2-1: The skull and upper limbs of a partially dislocated burial set in a north-south direction, with the top of the skull to the north. The rest of the skeleton was missing. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (4–5). Archaeological dating: Funnel Beaker culture, Siřem stage. Radiocarbon dating: PSUAMS-4235 (4770±20) 3638–3522 cal BC 2-sigma. (Not published, find reports 3730/83 and 2518/85). Master ID and/or other aDNA signs: I7194, MKTY_2–1. NM Prague, Inv. No.: P7A 38346/b.

Skeleton 3: partially dislocated burial in the north-south direction, with the top of the skull to the north. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: adultus II – maturus I (30–50). Archaeological dating: Funnel Beaker culture, Siřem stage. Radiocarbon dating: not available. (Not published, find reports 3730/83 and 2518/85). Master ID and/or other aDNA signs: I14173. NM Prague, Inv. No.: P7A 38346/e.

Skeleton 4: heavily dislocated burial with the skull facing east. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: adultus II – maturus I (30–50). Archaeological dating: Funnel Beaker culture, Siřem stage. Radiocarbon dating: not available. (Not published, find reports 3730/83 and 2518/85). Master ID and/or other aDNA signs: I16121. NM Prague, Inv. No.: P7A 38346/d.

Skeleton 6: left-sided crouched burial, head towards the south-east, with trunk turned on stomach. Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: adultus II – maturus I (30–50). Archaeological dating: Funnel Beaker culture, Siřem stage. Radiocarbon dating: not available. (Not published, find reports 3730/83 and 2518/85). Master ID and/or other aDNA signs: I16122. NM Prague, Inv. No.: P7A 38346/f.

22. Mikulovice (Mikulovice, Pardubice district, east Bohemia, Czech Republic)

Contact person: Michal Ernée

Rescue excavation in 2006–2012 (J. Frolík, R. Sedláček). Altogether, thousands of sunken settlement features and about 100 Early Bronze Age graves were documented in several groupings. With the exception of two Proto-Únětice graves (No. 95 and 96), the graves mostly belong to the Classic and Post-Classic stages of the Únětice culture after Moucha *(112)*. The inhumation burials are very rich in so-called "exotics", especially amber artefacts, which are present in 27 graves. The EBA cemetery has been completely analysed and published (*60*).

Grave 1. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – child, aDNA – F. Age: infans (5–6). Grave goods: two vessels, bronze eyelet pin, amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: CRL-19406 (3575±22) 2016–1880 cal BC 2-sigma *(60).* Pandora No.: MIB024. NM Prague Inv. No.: P7A 43100.

Grave 7. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: maturus I (35–50). Grave goods: three vessels, animal bone, shell. Archaeological dating: Classic Únětice culture. Radiocarbon dating: CRL-19411 (3562±22) 1976–1782 cal BC 2-sigma *(60).* Pandora No.: MIB028. NM Prague Inv. No.: P7A 43106.

Grave 17. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – juvenis, aDNA – F. Age: juvenis (14–16). Grave goods: one small vessel, two bronze pins, one of them an eyelet pin, two bronze earrings, two bronze ribbed bracelets, amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30461 (3416±25) 1860–1640 cal BC 2-sigma *(60).* Pandora No.: MIB034. NM Prague Inv. No.: P7A 43116.

Grave 51. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – child, aDNA – M. Age: infans (4–5). Grave goods: one vessel, two bronze arm-rings, belt ornament made of amber beads and amber ring. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30481 (3516±20) 1908–1766 cal BC 2-sigma *(60).* Pandora No.: MIB001. NM Prague Inv. No.: P7A 43179.

Grave 55. Skeleton: right-sided crouched burial, head towards the south-east. Sex: anthropology – F, aDNA – F. Age: adultus (20–25). Grave goods: two vessels, bronze eye-headed pin, two bronze earrings, necklace made of amber beads and bronze/copper spirals, awl made of animal bone. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30482 (3494±19) 1883–1753 cal BC 2-sigma; CRL-19334 (3548±31) 1973–1771 cal BC 2-sigma *(60).* Pandora No.: MIB002. NM Prague Inv. No.: P7A 43181.

Grave 62. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – M, aDNA – M. Age: adultus I (20–25). Grave goods: awl made of animal bone. Archaeological dating: Pre-Classic to Classic Únětice culture. Radiocarbon dating: MAMS-30484 (3503±18) 1888–1759 cal BC 2-sigma *(60).* Pandora No.: MIB004. NM Prague Inv. No.: P7A 43186.

Grave 64. Skeleton 64a: right-sided crouched burial, head towards the south-east. Sex: anthropology – F, aDNA – F. Age: senilis (over 60). Grave goods: two vessels and animal bones. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30485 (3498±19) 1879–1775 cal BC 2-sigma *(60).* Pandora No.: MIB005. NM Prague Inv. No.: P7A 43187.

Grave 68. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – juvenis, aDNA – F. Age: juvenis (15–16). Grave goods: two to three vessels, bronze pin, two bronze earrings, necklace made of bronze spirals. Archaeological dating: Classic Únětice culture. Radiocarbon dating: MAMS-30483 (3538±19) 1937–1776 cal BC 2-sigma *(60).* Pandora No.: MIB003. NM Prague Inv. No.: P7A 43185.

Grave 92

Skeleton 92a: right-sided crouched burial, head towards the south. Sex: anthropology – child, aDNA – M. Age: infans (2.5–3.5). Grave goods: one vessel. Archaeological dating: Pre-Classic to Classic Únětice culture. Radiocarbon dating: MAMS-30493 (3601±19) 2022–1900 cal BC 2-sigma *(60).* Pandora No.: MIS001. NM Prague Inv. No.: P7A 43201.

Skeleton 92b: right-sided crouched burial, head towards the south. Sex: anthropology – child, aDNA – M. Age: infans (6–8). Grave goods: one vessel. Archaeological dating: Pre-Classic to Classic Únětice culture. Radiocarbon dating: MAMS-30494 (3582±19) 2012–1887 cal BC 2-sigma *(60).* Pandora No.: MIS002. NM Prague Inv. No.: P7A 43202.

Grave 93. Skeleton: right-sided crouched burial, head towards the south-east. Sex: anthropology – M, aDNA – M. Age: adultus II (20–35). Grave goods: animal bone. Archaeological dating: Classic Únětice culture. Radiocarbon dating: CRL-20110 (3550±23) 1955–1776 cal BC 2-sigma *(60).* Pandora No.: MIS004. NM Prague Inv. No.: P7A 43217.

Grave 96. Skeleton: right-sided crouched burial, head towards the south-east. Sex: anthropology – M, aDNA – M. Age: senilis (over 60). Grave goods: no grave goods. Archaeological dating: Proto-Únětice culture. Radiocarbon dating: CRL-20112 (3833±27) 2456–2199 cal BC 2-sigma *(60).* Pandora No.: MIS006. NM Prague Inv. No.: P7A 43176.

Settlement pit 2217. Skeleton 97c: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – F, aDNA – F. Age: maturus II – senilis (over 55). Grave goods: no grave goods. Archaeological dating: Classic Únětice culture. Radiocarbon dating: CRL-20113 (3659±23) 2134–1953 cal BC 2-sigma *(60).* Pandora No.: MIG010. NM Prague Inv. No.: P7A 43214.

Settlement pit 2412. Skeleton 98: right-sided crouched burial, head towards the south. Sex: anthropology – F?, aDNA – F. Age: adultus I (20–30). Grave goods: no grave goods. Archaeological dating: Classic to Post-Classic Únětice culture. Radiocarbon dating: CRL-20114 (3658±24) 2134–1951 cal BC 2-sigma *(60).* Pandora No.: MIG011. NM Prague Inv. No.: P7A 43203.

Settlement pit 3/08. Skeleton 99: left-sided crouched burial, head towards the north. Sex: anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: no grave goods. Archaeological dating: Únětice culture. Radiocarbon dating: CRL-20115 (3533±32) 1947–1759 cal BC 2-sigma*(60).* Pandora No.: MIG012. NM Prague Inv. No.: P7A 43209.

23. Neratovice (Neratovice, Mělník district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský

An inhumation grave discovered in a trench dug for a sewer line at the local chemical factory (V. Spurný, Institute of Archaeology, Prague) in 1962.

Grave 1. Skeleton: right-sided crouched burial, head towards the south-west-south. Sex: archaeology – ?, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: chipped industry – two blades. Archaeological dating: Jordanów culture. Radiocarbon dating: MAMS-45790 (5284±27) 4231–4000 cal BC 2-sigma. (Not published). Pandora No.: NER001. NM Prague Inv. No.: P7A 33838.

24. Obříství (Obříství, Mělník district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Monika Pecinovská, Petra Stránská

Rescue excavation during the construction of roadways and utility lines for a new residential zone in 2011 (M. Pecinovská, Institute of Archaeology, Prague). The excavation area extended to ca. 3 ha and contained hundreds of settlement features (Neolithic, Bell Beaker culture, Bronze Age, Hallstatt period, Roman Iron Age) and the Corded Ware inhumation graves described below. The graves were 50 m from each other (grave 1 to grave 372), or 150 m from each other (grave 1 to grave 166). The site is probably part of a larger cemetery that has not yet been investigated. The described features remain unpublished; a preliminary report appears in *(156)*.

Grave 1. Grave with two skeletons.

Skeleton 1 (K1): left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus II – maturus I (35–50). Grave goods: two animal bone disks, bone awl/pin. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-30794 (4276±22) 2913–2882 cal BC 2-sigma (human bone); MAMS-41363 (4064±23) 2835–2492 cal BC 2-sigma (human bone); BRAMS–2959 (4016±26) 2617–2467 cal BC 2-sigma (animal bone disc 2). (Not published). Pandora No.: OBR001A, B. NM Prague Inv. No.: P7A 42983.

Skeleton 2 (K2). left(?)-sided crouched burial, head towards the east. Sex: archaeology – ?, anthropology – newborn, aDNA – F. Age: infans (newborn). Grave goods: without grave goods. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-38471 (3941±25) 2562–2345 cal BC 2-sigma (human bone); MAMS-41364 (3861±34) 2462–2209 cal BC 2-sigma (human bone). (Not published). Pandora No.: OBR002A. NM Prague Inv. No.: P7A 42984.

Grave 166. Skeleton: right-sided crouched burial, head towards the north-west. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: adultus II – maturus I (35–50). Grave goods: pot, two bone belt clasps, stone battle axe (A-type), chipped industry – blade. Archaeological dating: Corded Ware culture, early stage. Radiocarbon dating: MAMS-30795 (4259±23) 2911–2875 cal BC 2-sigma. (Not published). Pandora No.: OBR003A. NM Prague Inv. No.: P7A 42985.

Grave 372. Skeleton: left-sided crouched burial, head towards the east-south-east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: senilis (over 60). Grave goods: amphora, beaker, jar. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-38483 (4048±26) 2832–2482 cal BC 2-sigma. (Not published). Pandora No.: OBR004A, B. NM Prague Inv. No.: P7A 42986.

25. Plotiště nad Labem (Plotiště nad Labem, Hradec Králové district, east Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petra Stránská

Large-scale research (´systematic´) excavation by A. Rybová and V. Vokolek in 1961–1970. Its area, in front of a brickyard, covered ca. 1 ha. The site was multicultural, with the Stroked Pottery culture settlement and cemetery; Funnel Beaker, Bošáca, Únětice and Silesia-Platěnice culture settlement finds and a cremation cemetery from the Roman Iron Age). The lone Corded Ware culture grave, furnished with antler belt clasps (LX), was recently joined by another two graves (LIX and 221b) based on radiocarbon dating. These two graves had originally been regarded as Stroked Pottery graves (both inhumation graves without any additional finds). All have been published in detail; see *(157-160).*

Grave LX. Skeleton: right-sided crouched burial, head towards the north-west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus I (25–30). Grave goods: two bone belt clasps, bone awl, chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-41376 (4271±25) 2914–2879 cal BC 2-sigma *(157, 158).* Pandora No.: PNL001. NM Prague Inv. No.: P7A 36100.

Grave 221B. Skeleton: right-sided crouched burial, head towards the north-north-west. Sex: archaeology – M, anthropology – ?, aDNA – M. Age: juvenis (14–16). Grave goods: fragment of stone flat axe. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: Poz-86648 (4110±35), 2869–2573 cal BC 2 sigma *(159, 161).* Pandora No.: PNL002. NM Prague Inv. No.: P7A 43219.

26. Praha - Ďáblice (Ďáblice, Praha 8, central Bohemia, Czech Republic)

Contact persons: Michal Kostka, Lubor Smejtek, Michal Ernée

Excavated in 1993 during a rescue excavation (M. Kostka). A small cemetery from the early stage of the Únětice culture with 15 inhumation graves (14 in one group and one isolated grave). A total of 29 buried individuals were identified in the skeletal remains (7 men, 7 women, 9 children and 6 undeterminable). Multiple burials with two to five skeletons were also discovered. Ceramic vessels were mainly found as grave goods (one to five per grave). Two copper rings and some bone artefacts also sporadically occurred. The cemetery was completely analysed and published (*162*, *163*).

Grave 22B. Skeleton: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: two vessels. Archaeological dating: early stage of the Únětice culture (Early Únětice stage after periodisation of V. Moucha). Radiocarbon dating: MAMS-30755 (3675±22) 2137–1978 cal BC 2-sigma *(162, 163)*. Pandora No.: PDA001. City of Prague Museum.

Grave 22D. Inhumation grave containing one complete skeleton (1) and the anthropological remains of another four buried individuals (2–5). Archaeological dating: early stage of the Únětice culture (Early Únětice stage after periodisation of V. Moucha) *(162, 163)*.

Skeleton 1. Skeleton: right-sided crouched burial, head towards the south-south-west. Sex: anthropology – M, aDNA – M. Age: adultus II (30–40). Grave goods: two vessels, ornamented bone disk, bone awl, copper/bronze earring. Radiocarbon dating: MAMS-30756 (3720±21) 2197–2036 cal BC 2-sigma; KI-4454 (3740±45) 2289–2025 cal BC 2-sigma *(162, 163)*. Pandora No.: PDA002. City of Prague Museum.

Skeleton 2. Skeleton: isolated skull deposited by the southern part of the east side of the grave pit. Sex: anthropology – F, aDNA – F. Age: juvenis (15–20). Grave goods: no grave goods. Radiocarbon dating: MAMS-38479 (3765±24) 2284–2058 cal BC 2-sigma *(162, 163)*. Pandora No.: PDA003. City of Prague Museum.

Skeleton 3. Skeleton: skull and crossed extremities deposited by the south side of the grave pit, over the skull of skeleton No. 1. Sex: anthropology – F, aDNA – F. Age: maturus I (40–50). Grave goods: no grave goods. Radiocarbon dating: MAMS-30757 (3717±20) 2196–2036 cal BC 2-sigma *(162, 163)*. Pandora No.: PDA004. City of Prague Museum.

Skeleton 4. Skeleton: skull and some other bones deposited by the southern part of the west side of the grave pit. Sex: anthropology – M, aDNA – M. Age: maturus II (50–60). Grave goods: no grave goods. Radiocarbon dating: MAMS-38480 (3786±25) 2288–2141 cal BC 2-sigma *(162, 163)*. Pandora No.: PDA005. City of Prague Museum.

27.1. Praha - Jinonice (Jinonice, Praha 5, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Katarína Petriščáková

Rescue excavation due to the construction of the Nové Butovice metro station in 1983 (J. Havel, J. Kovářík). A multicultural site extending to ca. 2 ha. Among other finds (Stroked Pottery, Jordanów and Únětice culture graves), seven Corded Ware culture graves were investigated. Based on the finds, the graves can be attributed to a lesser extent to the middle (Group II) and mostly to the late stage of the Corded Ware culture (Group III after *(164)*). Given the poor condition of the bones, only one skeleton was subjected to aDNA analysis. The graves were comprehensively published in *(165)* (grave nos. 1–7).

Grave 3 (Buchv 3). Skeleton: left-sided crouched burial, head towards the east. In the grave together with a right (?)-side crouched burial, head towards the north-east (infans, 4–6). Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus (20–40). Grave goods: cylindrical beaker, two amphorae, two jugs. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(165).* Pandora No.: JIN001. NM Prague Inv. No.: P7A 39106.

27.2. Praha - Jinonice, Holman's garden centre (Jinonice, Praha 5, central Bohemia, Czech Republic)

Published in (5).

Rescue excavations during the construction of the Prague metro in 1984-1986. A cemetery of the early Únětice culture was discovered, with 29 graves and skeletal remains of 41 individuals (aDNA was analysed in eight cases; see below). However, the site was not excavated completely. Besides the numerically dominant Únětice culture component, a small number of graves from other periods were found: the Neolithic (three Stroked Pottery inhumation graves with five burials *(166)*, see grave 70) and the Eneolithic (unpublished). Besides an evaluation of the relevant anthropological material *(167)*, the Únětice culture finds have not been published in detail and are only mentioned in works *(168).* A monograph is being prepared (*K. Petriščáková*).

Grave 54. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – M?, aDNA – F. Age: maturus I (40–50). Grave goods: two jugs, small pot. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(5, 167).* Master ID and/or other aDNA signs: I7195, PRAJIN54. NM Prague Inv. No.: P7A 16062.

Grave 59. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: adultus II – maturus I (35–50). Grave goods: bronze hair ring, bone awl, chipped industry – blade. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(5, 167)*. Master ID and/or other aDNA signs: I7196, PRAJIN59. NM Prague Inv. No.: P7A 16066.

Grave 70 (?). Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – M?, aDNA – M. Age: adultus I (20–30). Grave goods: two cups, bowl. Archaeological dating: Stroked Pottery culture. Radiocarbon dating: PSUAMS-4344 (5660±25 BP) 4546–4451 cal BC 2-sigma *(5, 166, 167).* Master ID and/or other aDNA signs: I7197, PRAJIN77. NM Prague Inv. No.: P7A 16073.

Grave 82. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: adultus I (20–30). Grave goods: bowl. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: PSUAMS-3885 (3685±25 BP) 2189–1978 cal BC 2-sigma *(5, 167)*. Master ID and/or other aDNA signs: I7198, PRAJIN82. NM Prague Inv. No.: P7A 16075.

Grave 84. Grave with two burials.

Skeleton 1 (84-I): disturbed burial. Sex: archaeology – ?, anthropology – M?, aDNA – M. Age: adultus I (20–30). Grave goods: cup, jug, bowl, fragments of three other vessels. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: PSUAMS-3886 (3700±25 BP) 2196–1985 cal BC 2-sigma *(5, 167)*. Master ID and/or other aDNA signs: I7199, PRAJIN84 (1). NM Prague Inv. No.: P7A 16077.

Skeleton 2 (84–II): right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: maturus (40–60). Grave goods: see skeleton 1. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(5, 167)*. Master ID and/or other aDNA signs: I7200, PRAJIN84 (2). NM Prague Inv. No.: P7A 16078.

Grave 88. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: adultus II (30–40). Grave goods: two cups, bowl, fragments of two other vessels and bronze hair rings. This grave contained another inhumation burial (not analysed). Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(5, 167)*. Master ID and/or other aDNA signs: I7201, PRAJIN88. NM Prague Inv. No.: P7A 16085.

Grave 94. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: adultus I (20–30). Grave goods: cup, bowl, bronze hair rings, chipped industry – arrowhead. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(5, 167)*. Master ID and/or other aDNA signs: I7202, PRAJIN94. NM Prague Inv. No.: P7A 16091.

Grave 97. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: adultus I (20–30). Grave goods: cup, chipped industry.

Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(5, 167)*. Master ID and/or other aDNA signs: I7203, PRAJIN97. NM Prague Inv. No.: P7A 16094.

28. Praha - Jinonice, Butovická St. (Jinonice, Praha 5, central Bohemia, Czech Republic)

Published in (5).

A part of a Bell Beaker culture bi-ritual cemetery. In 2007, a section of the cemetery was investigated during the rescue excavation before the construction of a family house (trenches of the house) in Butovická St., Prague-Jinonice. Six of the seven excavated features were inhumations, the last one was a funerary feature interpreted as an incineration place. Based on the skeleton position, three of the buried individuals were male, two female and one indefinite. One of female burials (grave no. 4) was probably placed in a wooden chamber. Burial assemblages consisted mainly of undecorated pottery (1–3 vessels), accompanied in one case by a flint arrowhead, in another grave by 11 antler buttons (*169*).

Grave 1 (500/07). Skeleton: right-sided crouched burial, head towards the south-west. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus (25–35). Grave goods: two bowls. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2801 (3805±20 BP) 2297–2147 cal BC 2-sigma *(5, 169)*. Master ID and/or other aDNA signs: I4946. City of Prague Museum, Inv. No.: A 380 082–100.

Grave 3 (502/07). Skeleton: left-sideed crouched burial, head towards the north. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (7). Grave goods: jar. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2852 (3750±20 BP) 2273–2047 cal BC 2-sigma *(5, 169)*. Master ID and/or other aDNA signs: I4895. City of Prague Museum, Inv. No.: A 380 103–113.

Grave 4 (504/07). Skeleton: heavily disturbed, likely right-sided crouched burial, head towards the south-east. Sex: archaeology – F?, anthropology – F?, aDNA – M. Age: maturus II (50–60). Grave goods: two jars, bowl, two large round decorated antler buttons, nine small V-shaped antler buttons, animal bones in and outside the bowls (*Sus scrofa* cf. *domestica*). Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 169)*. Master ID and/or other aDNA signs: I4947. City of Prague Museum, Inv. No.: A 380 151–276.

Grave 5 (505/07). Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – child, aDNA – F. Age: infans (around 6). Grave goods: jar, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2853 (3785±20 BP) 2288–2142 cal BC 2-sigma *(5, 169)*. Master ID and/or other aDNA signs: I4896. City of Prague Museum, Inv. No.: A 380 277–286.

Grave 6 (507/07). Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – F, aDNA – M. Age: adultus II (30–40). Grave goods: jar, chipped industry – arrowhead. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2864 (3780±20 BP) 2286–2139 cal BC 2-sigma *(5, 169)*. Master ID and/or other aDNA signs: I5514. City of Prague Museum, Inv. No.: A 380 290–309.

29. Praha - Kobylisy, Ke Stírce St. (Kobylisy, Praha 8, central Bohemia, Czech Republic)

Published in (5).

A part of a Bell Beaker culture bi-ritual cemetery excavated in 2005/2006 during the rescue excavation before construction work on residential houses in Ke Stírce St. in Prague-Kobylisy (for preliminary information, see *(170).* Eleven excavated features were inhumations, the last one (grave no. 6) was a cremation grave, probably with the place of incineration. One of the inhumations is probably a secondary burial. Based on the skeleton position, six of the buried individuals were male, one female and five undefined. The female burial (grave no. 14) was probably placed in a wooden chamber. The burial assemblages consisted mainly of undecorated pottery (1–3 vessels) accompanied by three antler buttons and animal bones. Two graves (grave nos. 11 and 12) consisted completely archery equipment (arrowheads, wristguards and, in one case, an antler flint-knapping tool and probably bow-covering made of animal bones). Eight of the excavated skeletons (graves) were well-preserved and sampled for aDNA analysis (grave nos. 4, 8–14, see *(5)*). The cemetery is not yet published but is currently being prepared for publication. There were also two Stroked Pottery/Lengyel culture inhumation burials in a settlement pit (feature 552) and two Únětice culture skeletons in settlements pits (feature 515 and 541).

Grave 4 (528). Skeleton: left-sided crouched burial, heavily disturbed, head towards the north-east. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (5–6). Grave goods: jar, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2843 (3790±20 BP) 2289–2143 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4885. City of Prague Museum, No.: A46/2005.

Grave 8 (541). Skeleton: left-sided crouched burial, well-preserved with animal disturbance, head towards the north-east. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (9–13). Grave goods: bowl with animal bones inside. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2844 (3740±20 BP) 2205–2042 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4886. City of Prague Museum, No.: A46/2005.

Grave 9 (542). Skeleton: left-sided crouched burial, disturbance or secondary inhumation, head probably towards the north-east. A chop injury is visible on the head. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (7–8). Grave goods: bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2845 (3730±20 BP) 2201–2039 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4887. City of Prague Museum, No.: A46/2005.

Grave 10 (543). Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (6–7). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2846 (3700±20 BP) 2190–2029 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4888. City of Prague Museum, No.: A46/2005.

Grave 11 (544). Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: chipped industry – three arrowheads, stone wristguard, antler flint–knapping tool, bow–covering made of animal bones. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon

dating: PSUAMS-2847 (3765±20 BP) 2281–2062 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4889. City of Prague Museum, No.: A46/2005.

Grave 12 (545). Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: two cups, bowl, chipped industry – five arrowheads, stone wristguard. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available (*5*). Master ID and/or other aDNA signs: I4890. City of Prague Museum, No.: A46/2005.

Grave 13 (546). Skeleton: extended position with crouched hands on the shoulders, head towards the north-east. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus (20–40). Grave goods: bowl, chipped industry – two arrowheads. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2848 (3765±20 BP) 2281–2062 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4891. City of Prague Museum, No.: A46/2005.

Grave 14 (547). Skeleton: right-sided crouched burial, head towards the south-west, probably remnants of wooden chamber, bowl resting on stair. Sex: archaeology – F, anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: bowl, three small V-shaped antler buttons. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-2854 (3795±20 BP) 2291–2144 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4945. City of Prague Museum, No.: A46/2005.

Settlement feature (pit) 515. Skeleton: right(?)-sided crouched burial, orientation (?). Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: ? Grave goods: without directly related goods (probably only settlement discard from the fill of feature). Archaeological dating: Únětice culture, classic stage. Radiocarbon dating: PSUAMS-2842 (3480±20 BP) 1882–1745 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4884. City of Prague Museum, No.: A46/2005.

Settlement feature (pit) 551. Skeleton: right-sided crouched burial, orientation (?). Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: ? Grave goods: without directly related goods (probably only settlement discard from the fill of feature). Archaeological dating: Únětice culture, classic stage. Radiocarbon dating: PSUAMS-2849 (3475±20 BP) 1881–1701 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4892. City of Prague Museum, No.: A46/2005.

Settlement feature (pit) 552 with two burials (17 and 18).

Skeleton 17: irregular inhumation burial, adult. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: ? Grave goods: with the exception of one amphora (?), without directly related goods (only settlement discard from the fill of feature). Archaeological dating: Stroked Pottery/Lengyel culture. Radiocarbon dating: PSUAMS-2850 (5550±20 BP) 4449–4348 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4893. City of Prague Museum, No.: A46/2005.

Skeleton 18: only skull, child (?). Sex: archaeology – ?, anthropology – ?, aDNA – F. Age: ? Grave goods: see skeleton 17. Archaeological dating: Stroked Pottery/Lengyel culture.

Radiocarbon dating: PSUAMS-2851 (5610±20 BP) 4488–4368 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I4894. City of Prague Museum, No.: A46/2005.

30. Praha - Malá Ohrada (Stodůlky, Praha 5, central Bohemia, Czech Republic)

Rescue excavation due to the construction of the “Lužiny" prefab housing estate (J. Kovářík) in 1979–1980. Uncovered at the multicultural site with more than 500 features scattered over an area of ca. 1.5 ha was a great deal of evidence of settlement activities (Funnel Beaker culture, Late and Final Bronze Age, Hallstatt period, Roman Iron Age) and burials (Jordanów culture, Corded Ware culture, Bell Beaker culture, Únětice culture, Early Middle Ages), from which Corded Ware and Bellbeaker burials have been subjected to aDNA analysis thus far.

30.1. Praha - Malá Ohrada (CW)

Contact persons: Miroslav Dobeš, Katarína Petriščáková, Petr Velemínský

Of four investigated Corded Ware culture graves (grave nos. 53–56), only two were analysed for aDNA due to the poor condition of the bones. These were comprehensively published in *(165)*.

Grave 10 (Buchv 54). Skeleton: right-sided crouched burial, head towards the south-east. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: amphora, beaker, beaker with the handle, stone flat axe, copper artefact – knife?, whetstone, chipped industry – blade and flake. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-44709 (3987±25) 2571–2467 cal BC 2-sigma *(165).* Pandora No.: OHR001. NM Prague Inv. No.: P7A 38757.

Grave 26 (Buchv 55). Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: juvenis (14–15). Grave goods: two amphorae, beaker with handle, pot, copper spiral temple ring, two drilled shell discs, 275 drilled animal teeth and their imitations, 134 shell beads, 4 bone beads, chipped industry – blade and flakes. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-41374 (4017±25) 2579–2472 cal BC 2-sigma *(165).* Pandora No.: OHR002. NM Prague Inv. No.: P7A 38771.

30.2. Praha - Malá Ohrada (BB)

Published in (5).

An isolated Bell Beaker inhumation grave was investigated at the site in 1979. The grave has not yet been published in detail.

Grave 2 (507/07). Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – F?, aDNA – F. Age: adultus II – maturus I (35–50). Grave goods: unornamented bell beaker. Archaeological dating: Bell Beaker culture, middle stage. Radiocarbon dating: PSUAMS-4350 (3810±20 BP) 2334–2149 cal BC 2-sigma (*5*) Master ID and/or other aDNA signs: I7281, PRH5_2. NM Prague Inv. No.: P7A 38749.

31. Praha - Miškovice (Miškovice, Praha 9, central Bohemia, Czech Republic)

Contact person: Michal Ernée

In 1999 and 2001, a part of the inhumation cemetery of the EBA Únětice culture was investigated during the rescue excavation before the construction of family houses. Wooden coffins were identified in some graves, while more burials were positioned under stone constructions. Burial assemblages consisted mainly of undecorated pottery, accompanied by bronze (in 19 graves; pins, earrings, necklaces, daggers, axe) and amber artefacts (in 12 graves; beads, spacer), in three cases by seashells, etc. The earliest graves are dated to the Proto-Únětice culture (grave nos. 1–6, 13–15, 29, 39–40; ca. 2300–2150/2100 BC; stage Bz A0). The majority of the graves are dated to the Classic Únětice culture (graves 7–10, 16–28, 30–38, 41–44; ca. 2000–1850 BC; stage Bz A2a) The latest grave, no. 27 of the Post-Classic (Late) Únětice culture, contains a pin with a globular head. The cemetery was completely published (*114*, *171–174*).

Grave 4. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: adultus II – maturus I (35–50). Grave goods: three vessels. Archaeological dating: Proto-Únětice culture. Radiocarbon dating: KIA-35075 (3755±30) 2286–2038 cal BC 2-sigma; MAMS-19125 (3791±24) 2298–2138 cal BC 2-sigma *(114).* Pandora No.: PMI002. NM Prague Inv. No.: P7A 41112.

Grave 13. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: infans (11–13). Grave goods: one small vessel (jug) and one flint artefact. Archaeological dating: Proto-Únětice culture. Radiocarbon dating: KIA-30941 (3770±35) 2296–2039 cal BC 2-sigma; MAMS-19126 (3745±22) 2271–2042 cal BC 2-sigma *(114).* Pandora No.: PMI001. NM Prague Inv. No.: P7A 41108.

Grave 29. Skeleton: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – F, aDNA – F. Age: adultus I–II (25–40). Grave goods: three vessels (one Proto-Únětice jug) and two bronze earrings. Archaeological dating: Proto-Únětice culture. Radiocarbon dating: UtC-13189 (3670±35) 2192–1947 cal BC 2-sigma *(114).* Pandora No.: PMI003. NM Prague Inv. No.: P7A 41134.

Grave 39. Skeleton: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – M, aDNA – M. Age: maturus II – senilis (over 50). Grave goods: no grave goods. Archaeological dating: Proto-Únětice culture. Radiocarbon dating: KIA-35083 (3675±30) 2141–1956 cal BC 2-sigma *(114).* Pandora No.: PMI004. NM Prague Inv. No.: P7A 41141.

Grave 40. Skeleton: left-sided crouched burial, head towards the south-south-east. Sex: anthropology – ?, aDNA – M. Age: adultus I (20–30). Grave goods: one small jug in the grave pit fill. Archaeological dating: Proto-Únětice culture. Radiocarbon dating: KIA-35072 (3750±30) 2284–2037 cal BC 2-sigma *(114).* Pandora No.: PMI006. NM Prague Inv. No.: P7A 41142.

Grave 8. Skeleton: decomposed bones deposited on the bottom of the grave pit. Sex: anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: one small vessel, bronze eyelet pin of the Únětice type, two bronze earrings, necklace made of bronze spirals and amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: KIA-35076

(3615±30) 2118–1889 cal BC 2-sigma *(114).* Pandora No.: PMI011. NM Prague Inv. No.: P7A 41115.

Grave 18. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: juvenis (14–20). Grave goods: one small vessel, bronze knot-headed pin of the Cyprus type, three bronze earrings, necklace made of bronze spirals and amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UtC-13186 (3530±40) 2008–1744 cal BC 2-sigma; KIA-35077 (3595±30) 2033–1831 cal BC 2-sigma *(114).* Pandora No.: PMI008. NM Prague Inv. No.: P7A 41124.

Grave 31. Skeleton: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: bronze socketed pin. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UtC-13190 (3560±35) 2021–1773 cal BC 2-sigma *(114).* Pandora No.: PMI009. NM Prague Inv. No.: P7A 41135.

Grave 32. Skeleton: right-sided crouched burial, head towards the south-south-east. Sex: anthropology – F?, aDNA – M. Age: maturus II (50–60). Grave goods: bronze dagger, bronze chisel, two ornamented bronze eyelet pins of the Únětice type, four amber beads. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UtC-13191 (3520±35) 1941–1746 cal BC 2-sigma; KIA-35081 (3510±30) 1922–1746 cal BC 2-sigma; MAMS-19129 (3460±21) 1878–1695 cal BC 2-sigma *(114).* Pandora No.: PMI007. NM Prague Inv. No.: P7A 41136.

Grave 42. Skeleton: right-sided crouched burial, head towards the south-east. Sex: anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: one small vessel, bronze neck-eye pin, three bronze earrings, necklace made of bronze spirals, amber beads and one bone bead. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UtC-13192 (3560±40) 2026-1769 cal BC 2-sigma; KIA-35084 (3595±30) 2033-1831 cal BC 2-sigma *(114).* Pandora No.: PMI010. NM Prague Inv. No.: P7A 41145.

Grave 27. Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: infans (8–12). Grave goods: bronze pin with globular head. Archaeological dating: Post-Classic (Late) Únětice culture. Radiocarbon dating: UtC-13188 (3410±40) 1877–1564 cal BC 2-sigma *(114).* Pandora No.: PMI012. NM Prague Inv. No.: P7A 41133.

32. Praha - Nové Butovice (Stodůlky, Praha 5, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Katarína Petriščáková

Rescue excavation on the building place of the Nové Butovice prefab housing estate in 1986 (J. Kovářík). Among other things (Stroked Pottery culture settlement, Únětice and Bylany culture graves), 32 Corded Ware culture graves were also investigated at the multicultural site over an area of ca. 7 ha. Based on the finds, the graves can be attributed exceptionally to the middle (Group II) and in the vast majority to the late stage of the Corded Ware culture (Group III after *(164)*). DNA analyses were conducted only selectively due to the poor condition of the bones. The graves were comprehensively published in *(165)* (grave nos. 19–50).

Grave 29 (Buchv 38). Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – ?, aDNA – no aDNA. Age: adultus I (20–30). Grave goods:

amphora, beaker with the handle, stone battle axe, stone flat axe. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(165).* Pandora No.: BUT001. NM Prague Inv. No.: P7A 39067.

Grave 33 (Buchv 42). Skeleton: left (?)-sided crouched burial, head towards the east. Sex: archaeology – F?, anthropology – child, aDNA – F. Age: infans (6–7). Grave goods: amphora, pot, two cups, copper spiral temple ring, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(165).* Pandora No.: BUT002. NM Prague Inv. No.: P7A 39070.

Grave 35 (Buchv 44). Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – ?, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: amphora, cylindrical beaker, two stone flat axes, whetstone, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-41375 (3898±26) 2467–2300 cal BC 2-sigma *(165).* Pandora No.: BUT003. NM Prague Inv. No.: P7A 39072.

33. Praha - Ruzyně (Ruzyně, Praha 6, central Bohemia, Czech Republic)

Contact person: Milan Kuchařík

Rescue excavation due to the construction of family houses in 2011-2012 (J. Vávra, P. Zelená). Besides Eneolithic, Bronze Age (Knovíz) and Early Iron Age (Bylany) culture settlements and a grave from the La Tène period, seven inhumation graves of the Bell Beaker culture were also investigated at the multicultural site, six of which were analysed (see below). The cemetery has not been published in detail to date (for preliminary information, see *(175)*).

Grave 1 (feature 5097). Skeleton: right sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F, aDNA – F. Age: maturus II – senilis (over 50). Grave goods: bowl. Archaeological dating: Bell Beaker culture, early/middle phase. Radiocarbon dating: MAMS-38482 (3893±25) 2465–2299 cal BC 2-sigma. (Not published). Pandora No.: PRU001A. The City of Prague Museum, Inv. No.: A 628 926–937.

Grave 2 (feature 5004). Skeleton: right sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (8–9). Grave goods: decorated beaker. Archaeological dating: Bell Beaker culture, early/middle phase. Radiocarbon dating: MAMS-30800 (3995±23) 2571–2470 cal BC 2-sigma. (Not published). Pandora No.: PRU002A, B. The City of Prague Museum, Inv. No.: A 627 516–519.

Grave 3 (feature 5045). Skeleton: right sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F, aDNA – F. Age: maturus II (50–60). Grave goods: bowl, two cups. Archaeological dating: Bell Beaker culture, early/middle phase. Radiocarbon dating: MAMS-30801 (3957±24) 2567–2350 cal BC 2-sigma. (Not published). Pandora No.: PRU003A, B. The City of Prague Museum, Inv. No.: A 628 001–015.

Grave 4 (feature 5029). Skeleton: left sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: juvenis – adultus I (18–22). Grave goods: decorated handled beaker, cup. Archaeological dating: Bell Beaker culture, early/middle phase. Radiocarbon dating: MAMS-44707 (3848±24) 2456–2207 cal BC 2-sigma. (Not published). Pandora No.: PRU004.A, B. The City of Prague Museum, Inv. No.: A 627 695–703.

Grave 6 (feature 5087). Skeleton: right sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (10–12). Grave goods: cup Archaeological dating: Bell Beaker culture, early/middle phase. Radiocarbon dating: not available. (Not published). Pandora No.: PRU005A, B, C. The City of Prague Museum, Inv. No.: A 628 907–916.

34. Předměřice nad Labem (Předměřice nad Labem, Hradec Králové district, east Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský

The inadequately documented inhumation grave was investigated in the wall of the brickyard in 1921 (F. Žaloudek). This is essentially the same site as in Plotiště nad Labem (see above), since the two brickyards are adjacent to one another. Only one of the accompanying skulls reportedly spread around the skull of the complete burial in a crouched position could be subjected to aDNA analysis. It was published in detail in *(176, 177)*.

Collective grave without number.

Skeletons: right-sided crouched burial, head towards the north-east, and four skulls (one of them was analysed). Grave goods: amphora, jug. Archaeological dating: Globular Amphora culture, eastern group.

Skull. Sex: archaeology – ?, anthropology – F?, aDNA – F. Age: adult? (20–40?). Radiocarbon dating: MAMS-41368 (4095±26) 2858–2504 cal BC 2-sigma *(176, 177).* Pandora No.: PRE001. NM Prague Inv. No.: P7A 6867 (210c).

35 Radovesice (Radovesice, Teplice district, NW Bohemia, Czech Republic)

The cadastre area of the municipality, gradually destroyed by the activity of the open-cast brown coal mine, was investigated in the 1970s and 80s (J. Muška and P. Budinský, Teplice Museum). Evidence of settlement and burial activities from almost the whole prehistory was found at nearly twenty sites (Radovesice I to XIX). The Corded Ware and Bell Beaker graves described below were discovered at five sites. The sites were between 200 m to 1 km from one another.

35.1. Radovesice XVI

Contact persons: Miroslav Dobeš, Petr Velemínský

A Corded Ware grave was excavated by P. Budinský in 1983 at the “Pod Chlomkem” location and other features (mostly settlement features) from many other prehistoric periods (Neolithic, Funnel Beaker culture, Bronze Age, Hallstatt and La Tène period) were investigated at the site.

Grave 41/83, in the middle of a round ditch with a diameter of ca. 9 metres.

Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus II (50–60). Grave goods: stone battle axe, bone pin, chipped industry – blade. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-45791 (4081±25) 2852–2498 cal BC 2-sigma *(119).* Pandora No.: RDV001. NM Prague.

35.2. Radovesice III and XIII

Published in (5).

Bell Beaker culture graves were discovered in two positions approximately 800 m apart.

1. Two inhumation graves were explored in the "Za kostelem" site (Radovesice III) by J. Muška in 1978 (grave nos. 116 and 117/ 78). Both were richly equipped with vessels, copper daggers and an awl, stone wristguards and gold and silver jewellery. Both belong to the typologically earlier Bell Beaker culture.

2. Thirteen graves (here grave nos. 2, 53, 59, 67, 68, 69, 70, 71, 73 and 74/80), twelve inhumations and one cremation, were explored by J. Muška at the "U silnice do Kostomlat" site (Radovesice XIII). They were spread over an area of 100 x 25 m, but it was obviously only part of the cemetery. The vast majority of graves are of the typologically later Bell Beaker culture, for which is the incidence of so-called ´associated pottery´ is typical. Only selected graves are published (*(178)*, preliminary report *(179)*).

Grave 116/78. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II (30–40). Grave goods: decorated beaker, cup, copper dagger, stone wristguard, antler artefact, boar tooth, chipped industry. Archaeological dating: Bell Beaker culture, early stage. Radiocarbon dating: not available *(5, 178)*. Master ID and/or other aDNA signs: I7282, RDVS_116/78. NM Prague Inv. No.: P7A 9320.

Grave 117/78. Skeleton: right-sided crouched burial, head towards the north-east. Sex: archaeology – atypical (right side = female, orientation and grave goods = male), anthropology – F, aDNA – F. Age: maturus II – senilis (over 50). Grave goods: decorated beaker, decorated cup, gold and silver jewellery, copper dager, copper awl, chipped industry. Archaeological dating: Bell Beaker culture, early stage. Radiocarbon dating: KI-4448 (3860±45 BP) 2464–2205 cal BC 2-sigma *(5, 58, 178)*. Master ID and/or other aDNA signs: I7283, RDVS_117/78. NM Prague Inv. No.: P7A 9321.

Grave 2/80. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II (30–40). Grave goods: cup. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 178)*. Master ID and/or other aDNA signs: I7205, RDVS_02/80. NM Prague Inv. No.: P7A 9323.

Grave 53/80-I. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus I (20–30). Grave goods: beaker, cup, bowl, stone wristguard. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4352 (3830±20 BP) 2399–2201 cal BC 2-sigma; KI-4449 (3860±40 BP) 2464–2206 cal BC 2-sigma *(5, 58, 178)*. Master ID and/or other aDNA signs: I7286, RDVS_53/80-I. NM Prague Inv. No.: P7A 9325.

Grave 59/80-I. Disturbed inhumation burial. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (4–5). Grave goods: two cups, bowl, chipped industry. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available (*5*). Master ID and/or other aDNA signs: I7287, RDVS_59/80-I. NM Prague Inv. No.: P7A 9327.

Grave 59/80-II. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II (30–40). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available (*5*). Master ID and/or other aDNA signs: I7288, RDVS_59/80-II. NM Prague Inv. No.: P7A 9328.

Grave 67/80. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus I (40–50). Grave goods: none. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4353 (3860±20 BP) 2459–2214 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I7289, RDVS_67/80. NM Prague Inv. No.: P7A 9329.

Grave 68/80. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus I (35–50). Grave goods: none. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4236 (3730±20 BP) 2201–2039 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I7210, RDVS_68/80. NM Prague Inv. No.: P7A 9330.

Grave 69/80. Skeleton: right-sided crouched burial, head towards the south-west. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: adultus I (20–30). Grave goods: none. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4345 (3800±20 BP) 2293–2146 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I7211, RDVS_69/80. NM Prague Inv. No.: P7A 9331.

Grave 70/80. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (8–9). Grave goods: chipped industry. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4346 (3840±25 BP) 2456–2203 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I7212, RDVS_70/80. NM Prague Inv. No.: P7A 9332.

Grave 71/80. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: juvenis (15–18). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: PSUAMS-4347 (3835±20 BP) 2431–2202 cal BC 2-sigma (*5*). Master ID and/or other aDNA signs: I7213, RDVS_71/80. NM Prague Inv. No.: P7A 9333.

Grave 73/81. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: juvenis (17–20). Grave goods: V-bored amber buttons, amber beads. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available (*5*). Master ID and/or other aDNA signs: I7290, RDVS_73/81. NM Prague Inv. No.: P7A 17334.

Grave 74/81. Skeleton: right-sided crouched burial, head towards the south-east. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: juvenis – adultus I (15–25). Grave goods: two cups. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available (*5*). Master ID and/or other aDNA signs: I7214, RDVS_74/81. NM Prague Inv. No.: P7A 17335.

35.3-1. Radovesice III

Published in (61).

Grave 40/78 was excavated by J. Muška at the "Za kostelem" site.

Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: juvenis (18–20). Grave goods: beaker, amphora, stone flat axe, chipped industry – blade. Archaeological dating: Corded Ware culture, late stage. Radiocarbon dating: PSUAMS-3888 (3885±25 BP) 2464–2295 cal BC 2-sigma *(61, 119)*. Master ID and/or other aDNA signs: I7208, RDVS_40/78. NM Prague Inv. No.: P7A 9319.

35.3-2. Radovesice X

Published in (61).

Grave 5/79 was excavated by J. Muška at the "U bílinské silnice" site.

Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus I (40–50). Grave goods: beaker, amphora, jug, stone club and flat axe. Archaeological dating: Corded Ware culture, late stage. Radiocarbon dating: PSUAMS-4026 (3850±25 BP) 2458–2207 cal BC 2-sigma *(61, 119)* Master ID and/or other aDNA signs: I7209, RDVS_5/79. NM Prague Inv. No.: P7A 9322.

35.3-3. Radovesice XVIII

Published in (61).

Grave 4/81 was excavated by P. Budinský at the "Na vyhlídce" site.

Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus I (30–50). Grave goods: beaker, amphora, jug, another vessel, stone battle–axe and flat axe. Archaeological dating: Corded Ware culture, late stage. Radiocarbon dating: PSUAMS-3887 (3935±25 BP) 2559–2340 cal BC 2-sigma *(61, 119)*. Master ID and/or other aDNA signs: I7207, RDVS_04/81. NM Prague Inv. No.: P7A 17333.

36. Roudnice nad Labem (Litoměřice district, central Bohemia, Czech Republic)

Contact persons: Martin Trefný, Jana Kuljavceva Hlavová

In 2014, a collective inhumation grave from the early stage of early Únětice culture was uncovered during a rescue excavation prior to the construction of a private garden pool. Only one grave was uncovered. The grave was partially damaged by the scraper, causing two skulls to be extracted from the grave. Nevertheless, after the find context was cleaned, it was determined that a major part of the grave had not been affected by the activity of the scraper.

The grave pit had an irregular rectangular plan with a length of 2.11 m and a width of 1.0 to 1.05 m. The depth of the grave was 0.7 m.

Únětice collective grave, feature no. 1/2014

Skeletons: The grave contained more or less complete skeletal remnants of at least 18 individuals (ten of which were children) which were not oriented in anthropological positions. On the contrary, the remnants were mixed together, so that it was not possible to identify parts of the skeletons belonging to the individual burried persons. In every case it was evident at first glance that only parts of the bodies had been secondarily buried in the grave.

Grave goods: Eight vessels or remnants thereof were already clearly visible during the excavation and final preparation of the grave. The remnants of eight other vessels were discovered during the removal of the bones. The grave also contained a fragment of a bone needle, a fragment of a silex blade, three shells and three animal teeth, two of which were perforated.

Archaeological dating: stage 2 (Early Únětice) of the Únětice culture after Moucha *(112)*.

Skeleton (skull) 1. Sex: anthropology – F, aDNA – F. Age: maturus (over 40). Radiocarbon dating: not available. Pandora No.: ROU001. Roudnice nad Labem Museum.

Skeleton (skull) 2. Sex: anthropology – F, aDNA – F. Age: adultus II (30–40). Radiocarbon dating: MAMS-44708 (3662±25) 2134–1955 cal BC 2-sigma. Pandora No.: ROU002. Roudnice nad Labem Museum.

Skeleton (skull) 3. Sex: anthropology – child, aDNA – F. Age: infans (9 years ±24 months). Radiocarbon dating: not available. Pandora No.: ROU003. Roudnice nad Labem Museum.

Skeleton (skull) 4. Sex: anthropology – F, aDNA – F. Age: maturus I (40–50). Radiocarbon dating: not available. Pandora No.: ROU004. Roudnice nad Labem Museum.

Skeleton (skull) 5. Sex: anthropology – F, aDNA – M. Age: adultus II (30–40). Radiocarbon dating: not available. Pandora No.: ROU005. Roudnice nad Labem Museum.

Skeleton (skull) 6. Sex: anthropology – F, aDNA – F. Age: maturus (over 40). Radiocarbon dating: not available. Pandora No.: ROU006. Roudnice nad Labem Museum.

Skeleton (skull) 7. Sex: anthropology – M?, aDNA – M. Age: maturus I (40–50). Radiocarbon dating: not available. Pandora No.: ROU007. Roudnice nad Labem Museum.

37. Stadice (Stadice, Ústí nad Labem district, NW Bohemia, Czech Republic)

Contact person: Miroslav Dobeš

Excavated in 1987 during the rescue excavation prior to the construction of the D8 motorway (D. Koutecký, M. Cvrková). In addition to settlement features from the Early and Late Bronze Age, three Corded Ware culture inhumation graves were also uncovered at the site. Based on the finds, the graves date to the early period of this culture (Group I and II after *(164)*). Samples for aDNA analysis were taken from all of them, and their complete publication is found in *(180, 181)*.

Grave 28/87. Skeleton: right-sided crouched burial, head towards the west-north-west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus (20–40). Grave goods: beaker, bowl, stone club. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: not available *(180, 181).* Pandora No.: STD001. NM Prague Inv. No.: P7A 38886.

Grave 29/87. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (around 2). Grave goods: three shell beads, chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-45792 (4177±25) 2882–2673 cal BC 2-sigma *(180, 181).* Pandora No.: STD002. NM Prague Inv. No.: P7A 38885.

Grave 67/87. Skeleton: left-sided crouched burial, head towards the north-east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: maturus I (40–50). Grave goods: amphora, bone awl, chipped industry – blade. Archaeological dating: Corded Ware culture, early stage. Radiocarbon dating: MAMS-45793 (4314±25) 3010–2889 cal BC 2-sigma *(180, 181).* Pandora No.: STD003. NM Prague Inv. No.: P7A 38887.

38. Tišice (Tišice, Mělník district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Martin Kuna, Petra Stránská, Petr Velemínský

Rescue excavation in the sandpit area in 1996–2008 (D. Dreslerová, M. Kuna, P. Foster, J. Turek). The investigated area of ca. 25 ha produced settlement remains and burials from nearly all periods from the Neolithic up to the Early Middle Ages (a total of nearly 6,000 features), including the two Bell Beaker culture inhumation graves described below. They were discovered in different years (1999 and 2008), with a distance of ca. 450 between them. With the exception of a small amount of settlement evidence, no other artefacts of the discussed culture were found at the site. Only the first of the two inhumation graves (77/99) has been published in greater detail (*178*).

Grave 77/99. Skeleton: right-sided crouched burial, head towards the south-east. The same grave probably contained a secondary cremation burial. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: adultus II – maturus I (30–50). Grave goods: five decorated bell beakers, cup, pot, two gold hair ornaments, copper dagger, copper awl, two stone wristguards, amber V-perforated button, chipped industry – two flakes, bone artefact (?). Archaeological dating: Bell Beaker culture, early stage. Radiocarbon dating: MAMS-30796 (3887±25) 2464–2296 cal BC 2-sigma *(178).* Pandora No.: TIS001A, B. NM Prague Inv. No.: P7A 39900.

Grave 5707. Skeleton: right-sided crouched burial, head towards the south-east. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: adultus II – maturus I (35–50). Grave goods: decorated bell beaker and other four vessels, chipped industry – flake. Archaeological dating: Bell Beaker culture, early stage. Radiocarbon dating: MAMS-30797 (3995±23) 2571–2470 cal BC 2-sigma. (Not published). Pandora No.: TIS002A, B. NM Prague Inv. No.: P7A 42019.

39. Toušeň (Lázně Toušeň, Prague-East district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský, Jaroslav Špaček

Rescue excavation on the fortified hilltop "Hradišťko" settlement at the edge of the left-bank Elbe River terrace (J. Špaček) in 1975–1982, 1997 and 2000–2003. Among settlement and burial finds of the Únětice culture and later periods, numerous Řivnáč culture features were documented, including two inhumation burials and four semi-sunken huts with more human bones. Grave (burial) nr. 15 (see below) has not yet been published in detail but only mentioned in *(182, 183)*.

Grave No. 15. Skeleton: right sided crouched (?) burial, head towards to south-east. Found in a cultural layer, some parts of the skeleton were missing (lower limbs, pelvis). Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (12–18 month). Grave goods: miniature bottle-like vessel with four horns on the greatest diameter ("hanging bottle"). Archaeological dating: Řivnáč culture. Radiocarbon dating: MAMS-41357 (4166±24) 2878–2640 cal BC 2-sigma *(182, 183).* Pandora No.: TOU001. NM Prague Inv. No.: P7A 9271.

40. Trmice (Ústí nad Labem – Trmice, Ústí nad Labem district, NW Bohemia, Czech Republic)

Contact person: Miroslav Dobeš

Excavated in 1982 during the rescue excavation prior to the construction of a prefab concrete panel plant (M. Cvrková, grave no. 109/82 – TRM006) and the nearby D8 motorway in 1987 (D. Koutecký, M. Cvrková, other graves). Two Corded Ware culture graves were uncovered in 1982 (in addition to settlement features from the Late Bronze Age), in 1987 another 15 inhumation graves. Based on the accompanying finds (beakers, amphorae, stone battle-axes, stone flat axes, chipped flat axe, whetstones, chipped industry), all belong to the early phase of the Corded Ware culture (Group I and II after *(164)*), although a small number of the graves cannot be dated more precisely due to the absence of chronologically-sensitive artefacts (graves without finds, or containing only chipped industry. All of the graves were published in full in *(180, 184)*, including anthropological evaluations in *(181, 185)*.

Grave 4/87. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (6–7). Grave goods: stone battle axe, chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(180, 181).* Pandora No.: TRM001. NM Prague Inv. No.: P7A 38896.

Grave 8/87. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – no aDNA. Age: maturus I (40–50). Grave goods: beaker, stone battle axe, chipped flat axe. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: not available *(180, 181).* Pandora No.: TRM002. NM Prague Inv. No.: P7A 38900.

Grave 10/87. Skeleton: right-sided crouched burial, head towards the east (?). Sex: archaeology – M?, anthropology – M?, aDNA – F. Age: adultus I (20–30). Grave goods: stone battle axe, chipped industry – blade. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-45794 (4093±24) 2856–2506 cal BC 2-sigma *(180, 181).* Pandora No.: TRM003. NM Prague Inv. No.: P7A 38901.

Grave 16/87. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – child, aDNA – no aDNA. Age: infans (around 12). Grave goods: amphora,

beaker, chipped industry – two blades. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: not available *(180, 181).* Pandora No.: TRM005. NM Prague Inv. No.: P7A 38903.

Grave 109/82. Skeleton: right-sided crouched burial, head towards the west-south-west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus II (50–60). Grave goods: beaker, stone battle axe, chipped industry – blade. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-45796 (4105±25) 2859–2576 cal BC 2-sigma *(184, 185).* Pandora No.: TRM006. NM Prague Inv. No.: P7A 38608.

41. Tuchoměřice (Tuchoměřice, Prague-West district, central Bohemia, Czech Republic)

Contact person: Miroslav Dobeš, Lucia Mattiello, Petra Stránská

Excavated in 1997–1998, 2000 and 2010–2012 during the rescue excavation prior to the construction of warehouse halls, over a total area of ca. 7 ha (I. Pleinerová, A. Veselá, P. Sankot, L. Šulová). A multicultural site (Linear Pottery, Jordanów, Řivnáč culture settlement, Middle Bronze Age, Hallstatt period and Early La Tène), from which two features with the occurrence of human bones are described below. The first of these was a Jordanów culture inhumation burial interpreted as a construction offering, the second a semi-sunken hut of the Řivnáč culture secondarily used for the deposition of six human bodies. These were not accompanied by any grave goods. Besides the skeletons, the hut fill contained common Řivnáč culture settlement discard (pottery, daub, animal bones, chipped industry, etc.). The two features were situated ca. 300 m from each other. The Jordanów culture grave was published in detail (*186*, *187*), whereas only a preliminary report has been issued on the Řivnáč culture hut (*188*).

Feature No. 3. Possibly a foundation sacrifice. Skeleton: disturbed burial in an elongated pit with a north-south longitudinal axis. Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (around 12). Grave goods: jug, fragment of stone axe. Archaeological dating: Jordanów culture. Radiocarbon dating: MAMS-41356 (5288±25) 4231–4004 cal BC 2-sigma *(186, 187).* Pandora No.: TUC007. NM Prague Inv. No.: P7A 17395.

Feature 645. Six irregular inhumation burials in a semi–sunken hut. Without directly related goods (only settlement discard from the fill of the feature).

Skeleton/burial 1 (child 1). Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (5–7). Archaeological dating: Řivnáč culture. Radiocarbon dating: not available *(188).* Pandora No.: TUC001. NM Prague Inv. No.: P7A 43315.

Skeleton/burial 2 (child 2). Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (3–5). Archaeological dating: Řivnáč culture. Radiocarbon dating: MAMS-30752 (4378±22) 3085–2916 cal BC 2-sigma *(188).* Pandora No.: TUC002. NM Prague Inv. No.: P7A 43316.

Skeleton/burial 3 (child 3). Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (3–5). Archaeological dating: Řivnáč culture. Radiocarbon dating: not available *(188).* Pandora No.: TUC003. NM Prague Inv. No.: P7A 43317.

Skeleton/burial 4 (child 4). Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (2–4). Archaeological dating: Řivnáč culture. Radiocarbon dating: not available *(188).* Pandora No.: TUC004. NM Prague Inv. No.: P7A 43318.

Skeleton/burial 5 (adultus). Sex: archaeology – ?, anthropology – M, aDNA – M. Age: adultus II – maturus I (35–45). Archaeological dating: Řivnáč culture. Radiocarbon dating: MAMS-30753 (4386±22) 3089–2918 cal BC 2-sigma *(188).* Pandora No.: TUC005. NM Prague Inv. No.: P7A 43313.

Skeleton/burial 6 (child 5). Sex: archaeology – ?, anthropology – child, aDNA – F. Age: infans (9–11). Archaeological dating: Řivnáč culture. Radiocarbon dating: MAMS-30754 (4318±23) 3010–2891 cal BC 2-sigma *(188).* Pandora No.: TUC006. NM Prague Inv. No.: P7A 43314.

42.1. Velké Přílepy (Velké Přílepy, Prague-West district, central Bohemia, Czech Republic)

Contact persons: Miroslav Dobeš, Petr Velemínský

Excavated in 1994–1996 during the rescue excavation prior to the construction of 485 family homes, on a total area of ca. 10 ha (I. Vojtěchovská, L. Smejtek). This multicultural site was used with breaks for settlement and burials from the Eneolithic up to the early medieval period (c. 3700 BC to 1000 AD), including in the period of the Řivnáč culture, which is represented by four settlement features. In one of them (feature 193/95, see below), a human skeleton also accompanied common settlement discard (pottery, daub, animal bones, etc.). The Řivnáč culture settlement has not yet been published in detail, only as a preliminary report in *(189)*.

Feature No. 193, settlement pit. Skeleton: irregular inhumation burial. Sex: archaeology – ?, anthropology – child, aDNA – M. Age: infans (12–15). Without directly related goods (only settlement discard from the fill of the feature). Archaeological dating: Řivnáč culture. Radiocarbon dating: MAMS-41373 (4213±25) 2898–2698 cal BC 2-sigma *(189).* Pandora No.: VPR001. NM Prague Inv. No.: P7A 16353.

42.2. Velké Přílepy (Velké Přílepy, Prague-West district, central Bohemia, Czech Republic)

Published in (4, 5).

The graves described below also come from the same site of Velké Přílepy. The first two samples (from grave nos. 182 and 185) belong to the Bell Beaker culture, the cemetery of which at the site was composed of eight inhumation graves in two groups, all of which can be attributed to the late stage of the discussed culture. One sample (grave no. 238) belongs to the early stage of the Únětice culture (a total of eight graves from this period were investigated at the site). The archaeological context was also preliminarily published in *(189)*. Three graves with bell beakers were analysed for the occurrence of strontium (nos. 143, 185 and 188), including two described below *(138).*

Grave 185. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus I (40–50). Grave goods: without grave goods. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 189)*. Master ID and/or other aDNA signs: I6480, F0551. NM Prague Inv. No.: P7A 16350.

Grave 188. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (13–14). Grave goods: two cups and another vessel. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(5, 189)*. Master ID and/or other aDNA signs: I6468, F0553. NM Prague Inv. No.: P7A 16351.

Grave 238. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – ?, anthropology – M, aDNA – F. Age: maturus I (40–50). Grave goods: one vessel. Archaeological dating: Únětice culture, early stage. Radiocarbon dating: not available *(4, 5, 189)* – known in *(5)* as I5035. Master ID and/or other aDNA signs: I4139, RISE577, F0565. NM Prague Inv. No.: P7A 16359.

43. Velké Žernoseky (Velké Žernoseky,Teplice district, NW Bohemia, Czech Republic)

Published in (61).

The site is located on the right-bank terrace of the Elbe River and was explored at the turn of the 20th century. The burial grounds were damaged by the mining of porphyry. In addition to an isolated Corded Ware grave (see below), several dozen Funnel Beaker culture (c. 3700–3500 BCE) and Únětice culture (2200–1700 BC) graves were also found at the site.

Grave 27. Skeleton: crouched burial with a southeast-northwest orientation. Only the skull has been preserved from the skeleton. Sex: archaeology – ?, anthropology – M, aDNA – M. Age: maturus II – senilis (over 50). Grave goods: two antler belt clasps, bone pin. Archaeological dating: Corded Ware culture, early stage. Radiocarbon dating: MAMS-44710 (4127±25) 2866–2583 cal BC 2-sigma *(61, 118, 190)*. Master ID and/or other aDNA signs: I6696, VEZE_27. NM Prague Inv. No.: P7A 6589.

44. Vliněves (Mělník district, central Bohemia, Czech Republic)

Contact persons: Petr Limburský, Miroslav Dobeš, Petr Velemínský

Excavated in 1999–2008 during the rescue fieldworks in the area of a large sandpit (V. Salač, I. Pleinerová, Ž. Brnić, P. Limburský). An area of ca. 70 ha was investigated, with 30 ha providing traces of settlement activities and burials from many periods of prehistory and the Early Middle Ages. The earliest of them is a fortified Jordanów culture settlement with human burials in a ditch, followed by two graves from the Late Michelsberg/Early Baalberge, a Baden and Řivnáč culture settlement and one Globular Amphora culture (?) collective grave. The Corded Ware culture cemetery is composed of 75 inhumation graves (early to late stage), the Bell Beaker culture cemetery has 34 inhumation graves (late stage) and the Únětice culture cemeteries 304 inhumation graves (primarily from the classic period of the given culture). Settlement traces of varying intensity of the last three mentioned archaeological cultures were also identified at the site, i.e., the Corded Ware culture (only intrusions in later features), the Bell Beaker culture (two pits and intrusions in later features) and the Únětice culture (remains of multiple post houses and more than one hundred sunken features with the remains of 57 human skeletons). Finds of the following occupation of the site are represented by the Hallstatt and La Tène period settlements and 21 Migration Period graves.

One inhumation burial from the Jordanów culture ditch, a collective Globular Amphora culture grave (?) and selected human remains from Bell Beaker and Únětice culture graves were chosen for aDNA analysis. Samples were taken from all the skeletons of the Corded Ware culture for the given analysis (or as permitted by the state of preservation). With the exception of the Globular Amphora culture collective grave (feature 3512), the archaeological sources for the studied period were published in full in *(116, 191–193)*; for a short review, see *(194)*.

44.1. Vliněves – Eneolithic Pre-Corded Ware cultures

Grave 2633A. Skeleton: left-sided crouched burial, head towards the west. Sex: archaeology – ?, anthropology – F, aDNA – no aDNA. Age: adultus II – maturus (30–60). Grave goods: bowl. Archaeological dating: Funnel Beaker culture, Michelsberg/Baalberge–stage. Radiocarbon dating: KIA-40230 (5023±28) 3942–3712 cal BC 2-sigma *(192).* Pandora No.: VLI005. NM Prague Inv. No.: P7A 41384.

Grave 2895. Skeleton: left-sided crouched burial, head towards the west-north-west. Sex: archaeology – ?, anthropology – M?, aDNA – M. Age: adultus II – maturus (30–60). Grave goods: no finds. Archaeological dating: Funnel Beaker culture, Michelsberg/Baalberge stage. Radiocarbon dating: MAMS-41358 (4949±25) 3778–3660 cal BC 2-sigma *(192).* Pandora No.: VLI006. NM Prague Inv. No.: P7A 41400.

Grave 3512. Collective grave with three burials. Grave goods: one jug. Archaeological dating: Globular Amphora culture (??).

Skeleton 1: right-sided crouched burial, head towards the east. Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: adultus I – maturus I? (25–50?). Radiocarbon dating: CRL-10178 (3971±134) 2881–2138 cal BC 2-sigma; MAMS-41361 (4046±27) 2831–2479 cal BC 2-sigma. (Not published). Pandora No.: VLI031. NM Prague Inv. No.: P7A 41406.

Skeleton 2: left-sided crouched burial, head towards the east-south-east. Sex: archaeology – ?, anthropology – M?, aDNA – M. Age: adultus II – maturus I (30–50). Radiocarbon dating: MAMS-41362 (4132±24) 2871–2587 cal BC 2-sigma. (Not published). Pandora No.: VLI032. NM Prague Inv. No.: P7A 41407.

Skeleton 3: right-sided crouched burial, head towards the east-south-east. Sex: archaeology – ?, anthropology – F?, aDNA – M. Age: adultus – maturus (over 30). Radiocarbon dating: MAMS-38477 (4133±27) 2871–2589 cal BC 2-sigma. (Not published). Pandora No.: VLI033. NM Prague Inv. No.: P7A 41408.

Feature No.10589/9969 (ditch/causewayed enclosure). Skeleton: right-sided, subtly crouched burial, partially in the prone position, on the bottom of ditch, on its lengthwise axis. Sex: archaeology – ?, anthropology – F, aDNA – F. Age: maturus II – senilis (over 50). Without directly related goods (only settlement discard from the filling of feature). Archaeological dating: Jordanów culture. Radiocarbon dating: KIA-40232 (5357±27) 4324–4055 cal BC 2-sigma *(191).* Pandora No.: VLI004. NM Prague Inv. No.: P7A 41992.

44.2. Vliněves – Corded Ware culture

Grave 774. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: maturus? (40–60). Grave goods: amphora. Archaeological dating: Corded Ware culture, early stage. Radiocarbon dating: MAMS-30758 (4196±21) 2889–2695 cal BC 2-sigma *(192).* Pandora No.: VLI007. NM Prague Inv. No.: P7A 40934.

Grave 865. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (8–10). Grave goods: drilled animal tooth, shell artefact. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-45798 (4171±26) 2880–2640 cal BC 2-sigma *(192).* Pandora No.: VLI071. NM Prague Inv. No.: P7A 40937.

Grave 890. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: adultus II – maturus (30–60). Grave goods: amphora, chipped industry – ten flakes (partial intrusion?). Archaeological dating: Corded Ware culture, early stage. Radiocarbon dating: MAMS-30759 (4212±21) 2894–2703 cal BC 2-sigma *(192).* Pandora No.: VLI008. NM Prague Inv. No.: P7A 40938.

Grave 957. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus II – maturus (30–60). Grave goods: chipped industry – two flakes. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9182 (4261±118) 3330–2497 cal BC 2-sigma *(192).* Pandora No.: VLI072. NM Prague Inv. No.: P7A 40939.

Grave 965. Skeleton: left-sided crouched burial, head towards the south-east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: chipped industry – blade, pebble artefact (intrusion). Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9183 (3954±81) 2848–2201 cal BC 2-sigma; MAMS-45797 (4078±25) 2850–2497 cal BC 2-sigma *(192).* Pandora No.: VLI009. NM Prague Inv. No.: P7A 40940.

Grave 1045. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – ?, aDNA – no aDNA. Age: maturus (40–60). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9184 (4190±100) 3012–2490 cal BC 2-sigma *(192).* Pandora No.: VLI073. NM Prague Inv. No.: P7A 40956.

Grave 1070. Skeleton: right-sided crouched burial, head towards the west–north–west. Sex: archaeology – M, anthropology – ?, aDNA – no aDNA. Age: juvenis (15–19). Grave goods: chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(192).* Pandora No.: VLI074. NM Prague Inv. No.: P7A 40990.

Grave 1071. Skeleton: right-sided crouched burial, head towards the west–north–west. Sex: archaeology – M, anthropology – ?, aDNA – M. Age: juvenis – adultus I (15–30). Grave goods: fragment of beaker (?). Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(192).* Pandora No.: VLI075. NM Prague Inv. No.: P7A 40989.

Grave 1113. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M?, aDNA – no aDNA. Age: adultus? (20–40). Grave goods: amphora, jug, another vessel, stone battle axe, stone flat axe. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI066. NM Prague Inv. No.: P7A 41008.

Grave 1423. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (3–5). Grave goods: two drilled animal teeth, 25 shell beads. Archaeological dating: Corded Ware culture, aceramic/middle stage. Radiocarbon dating: MAMS-38472 (4027±25) 2618–2475 cal BC 2-sigma *(192).* Pandora No.: VLI010. NM Prague Inv. No.: P7A 41010.

Grave 1494. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: juvenis – adultus I (16–25). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9188 (4288±79) 3308–2624 cal BC 2-sigma; MAMS-41360 (4173±24) 2880–2671 cal BC 2-sigma *(192).* Pandora No.: VLI067. NM Prague Inv. No.: P7A 41014.

Grave 1515. Skeleton: left-sided crouched burial, head towards the east-north-east. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: maturus (40–60). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9189 (4225±81) 3015–2579 cal BC 2-sigma; MAMS-45799 (4340±25) 3018–2901 cal BC 2-sigma *(192).* Pandora No.: VLI076. NM Prague Inv. No.: P7A 41018.

Grave 1653. Skeleton: left-sided crouched burial, head towards the east-north-east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9190 (4261±88) 3261–2578 cal BC 2-sigma *(192).* Pandora No.: VLI077. NM Prague Inv. No.: P7A 41019.

Grave 2283. Skeleton: disturbed, position unknown. Sex: archaeology – ?, anthropology – child, aDNA – no aDNA. Age: infans (1–6). Grave goods: amphora, beaker (?), cylindrical beaker, bowl, miniature vessel. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI078. NM Prague Inv. No.: P7A 41067.

Grave 2583. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – child, aDNA – F. Age: infans (5–7). Grave goods: no finds. Archaeological dating: Corded Ware culture (?), aceramic. Radiocarbon dating: MAMS-47026 (4095±23) 2853-2503 cal BC 2-sigma *(192).* Pandora No.: VLI079. NM Prague Inv. No.: P7A 41096.

Grave 2699. Skeleton: left-sided crouched burial, head towards the east-south-east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: ? Grave goods: chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(192).* Pandora No.: VLI080. NM Prague Inv. No.: P7A 41398.

Grave 2891. Skeleton: right-sided crouched burial, head towards the west-north-west. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (7–13). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(192).* Pandora No.: VLI081. NM Prague Inv. No.: P7A 41104.

Grave 2898–1 (upper grave, probably earlier). Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – ?, aDNA – no aDNA. Age: maturus? (40–60). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: not available *(192).* Pandora No.: VLI082. NM Prague Inv. No.: P7A 41106.

Grave 2898–2 (lower grave, probably later). Skeleton: left-sided crouched burial, head towards the south-east. Sex: archaeology – F, anthropology – F?, aDNA – no aDNA. Age:

adultus (20–40). Grave goods: four bone beads, chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9192 (4226±95) 3089–2497 cal BC 2-sigma *(192).* Pandora No.: VLI083. NM Prague Inv. No.: P7A 41107.

Grave 3935. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – ?, aDNA – no aDNA. Age: adultus I (20–30). Grave goods: amphora, beaker, stone club, chipped industry – blade and flake. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI084. NM Prague Inv. No.: P7A 41427.

Grave 4214A. Skeleton: right-sided crouched burial, head towards the north-west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9194 (4133±87) 2896–2488 cal BC 2-sigma; MAMS-44711 (4174±25) 2881–2669 cal BC 2-sigma *(192).* Pandora No.: VLI011. NM Prague Inv. No.: P7A 41603.

Grave 4291. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: amphora, cup, pot, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI012. NM Prague Inv. No.: P7A 41441.

Grave 4295. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: amphora, pot, bowl, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI013. NM Prague Inv. No.: P7A 41442.

Grave 4307. Skeleton: right-sided crouched burial, head towards the west-north-west. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (3–5). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: MAMS-45800 (4110±27) 2862–2576 cal BC 2-sigma *(192).* Pandora No.: VLI085. NM Prague Inv. No.: P7A 41446.

Grave 4322. Skeleton: left-sided crouched burial, head towards the east-south-east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: amphora, pot, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI086. NM Prague Inv. No.: P7A 41448.

Grave 4391. Skeleton: left-sided crouched burial, head towards the south-east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: amphora, beaker with the handle, two jars, bowl, another vessel, chipped industry – blade. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI068. NM Prague Inv. No.: P7A 41454.

Grave 4398. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: amphora, jug. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(192).* Pandora No.: VLI014. NM Prague Inv. No.: P7A 41477.

Grave 4406. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: juvenis – adultus I (15–30). Grave goods: amphora, beaker with the handle, whetstone. Archaeological dating: Corded Ware culture, local (late) stage (?). Radiocarbon dating: not available *(192).* Pandora No.: VLI087. NM Prague Inv. No.: P7A 41566.

Grave 4584. in the middle of a circular ditch. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – child, aDNA – no aDNA. Age: infans (2–4). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: KIA-40231 (4125±26) 2866–2582 cal BC 2-sigma *(192).* Pandora No.: VLI069. NM Prague Inv. No.: P7A 41481.

Grave 4757. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus – senilis (over 40). Grave goods: chipped industry – two blades. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9198 (4247±80) 3085–2581 cal BC 2-sigma *(192).* Pandora No.: VLI015. NM Prague Inv. No.: P7A 41701.

Grave 4871. Skeleton: left-sided, subtly crouched burial (prone position), head towards the west (anomalous position). Sex: archaeology – ?, anthropology – ?, aDNA – M. Age: juvenis (15–18). Grave goods: amphora, stones and animal bone – intrusion. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-38473 (3913±26) 2472–2303 cal BC 2-sigma *(192).* Pandora No.: VLI016. NM Prague Inv. No.: P7A 41706.

Grave 5358. Skeleton: left-sided crouched burial, head towards the south–east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: adultus – maturus (20–60). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9199 (4217±92) 3081–2498 cal BC 2-sigma *(192).* Pandora No.: VLI088. NM Prague Inv. No.: P7A 41715.

Grave 5379. Skeleton: left-sided crouched burial, head towards the east-south-east. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: adultus (20–40). Grave goods: amphora, two jars, chipped industry – blade. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-38474 (3902±28) 2469–2300 cal BC 2-sigma *(192).* Pandora No.: VLI017. NM Prague Inv. No.: P7A 41718.

Grave 5432. Skeleton: right-sided crouched burial (disturbed), head towards the west. Sex: archaeology – M, anthropology – ?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9200 (4256±82) 3089–2583 cal BC 2-sigma *(192).* Pandora No.: VLI089. NM Prague Inv. No.: P7A 41720.

Grave 5790. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus II? (30–40). Grave goods: amphora (fragment). Archaeological dating: Corded Ware culture, local (late) stage (?). Radiocarbon dating: CRL-9201 (3961±99) 2861–2148 cal BC 2-sigma; MAMS-41359 (3776±23) 2286–2138 cal BC 2-sigma; MAMS-46362 (3918±21) 2473–2311 cal BC 2-sigma *(192).* Pandora No.: VLI070. NM Prague Inv. No.: P7A 41737.

Grave 6094. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: adultus – maturus (20–60). Grave goods: amphora,

beaker, jug, pot. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-30760 (3941±21) 2557–2346 cal BC 2-sigma *(192).* Pandora No.: VLI018. NM Prague Inv. No.: P7A 41743.

Grave 7473. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – ?, aDNA – F. Age: maturus – senilis (over 40). Grave goods: bone awl, chipped industry – flake. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9203 (4241±84) 3085–2578 cal BC 2-sigma *(192).* Pandora No.: VLI090. NM Prague Inv. No.: P7A 41750.

Grave 7520. Skeleton: left-sided crouched burial, head towards the south–east. Sex: archaeology – F, anthropology – F, aDNA – no aDNA. Age: adultus (20–40). Grave goods: no finds. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-9204 (4184±82) 2922–2495 cal BC 2-sigma *(192).* Pandora No.: VLI091. NM Prague Inv. No.: P7A 41751.

Grave 8171A. Skeleton: right-sided crouched burial, head towards the west. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus II – maturus (30–60). Grave goods: beaker, whetstone. Archaeological dating: Corded Ware culture, middle stage. Radiocarbon dating: MAMS-38475 (not enough collagen) *(192).* Pandora No.: VLI019. NM Prague Inv. No.: P7A 41802.

Grave 9566A. Skeleton: right-sided crouched burial, head towards the west-north-west. Sex: archaeology – M, anthropology – M?, aDNA – M. Age: maturus? (40–60). Grave goods: chipped industry – blade. Archaeological dating: Corded Ware culture, aceramic. Radiocarbon dating: CRL-10180 (4057±87) 2881–2349 cal BC 2-sigma; MAMS-45801 (4176±26) 2882–2669 cal BC 2-sigma *(192).* Pandora No.: VLI092. NM Prague Inv. No.: P7A 41821.

Grave 9730. Skeleton: left-sided crouched burial, head towards the east. Sex: archaeology – F, anthropology – F, aDNA – F. Age: adultus I (20–30). Grave goods: two amphorae, jug, cup, two drilled shell disks, bone awl. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: MAMS-30761 (3966±25) 2570–2353 cal BC 2-sigma *(192).* Pandora No.: VLI020. NM Prague Inv. No.: P7A 41824.

44.3. Vliněves – Bell Beaker culture

Grave 4333/H225. Skeleton: disturbed inhumation burial. Sex: archaeology – ?, anthropology – F?, aDNA – no aDNA. Age: adultus I (20–30). Grave goods: cup, fragments of another cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30762 (3815±21) 2338–2152 cal BC 2-sigma *(193).* Pandora No.: VLI021. NM Prague Inv. No.: P7A 41680.

Grave 4335/H226. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – F?, aDNA – F. Age: juvenis – adultus I (15–30). Grave goods: cup, handled pot. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30763 (3848±26) 2456–2207 cal BC 2-sigma *(193).* Pandora No.: VLI022. NM Prague Inv. No.: P7A 41479.

Grave 4339/H227. Skeleton: left-sided crouched burial, head towards the north-east-north. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: adultus? (20–40?). Grave

goods: no finds. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(193).* Pandora No.: VLI023. NM Prague Inv. No.: P7A 41681.

Grave 4340/H228. Skeleton: left-sided crouched burial, head towards the north-north-east. Sex: archaeology – M, anthropology – child, aDNA – M. Age: infans (9–12). Grave goods: no finds. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(193).* Pandora No.: VLI024. NM Prague Inv. No.: P7A 41449.

Grave 4392/H234. Skeleton: left-sided crouched burial, head towards the north-east-north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: maturus (40–60). Grave goods: cup. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(193).* Pandora No.: VLI025. NM Prague Inv. No.: P7A 41455.

Grave 4464/H244. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – M??, aDNA – F. Age: adultus I (20–30). Grave goods: cup, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(193).* Pandora No.: VLI026. NM Prague Inv. No.: P7A 41570.

Grave 4467/H245. Skeleton: right-sided crouched burial, head towards the south. Sex: archaeology – F, anthropology – ?, aDNA – no aDNA. Age: ? Grave goods: two cups, handled pot. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30764 (no collagen) *(193).* Pandora No.: VLI027. NM Prague Inv. No.: P7A 41572.

Grave 4468/H246. Skeleton: right-sided crouched burial (?), head towards the south. Sex: archaeology – F?, anthropology – ?, aDNA – M. Age: adultus II – maturus? (30–60). Grave goods: two cups, bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: MAMS-30765 (no collagen) *(193).* Pandora No.: VLI028. NM Prague Inv. No.: P7A 41573.

Grave 4471/H248. Skeleton: left-sided crouched burial, head towards the north-east-north. Sex: archaeology – M, anthropology – M, aDNA – M. Age: adultus I (20–30). Grave goods: bowl. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(193).* Pandora No.: VLI029. NM Prague Inv. No.: P7A 41574.

Grave 4475/H251. Skeleton: left-sided crouched burial, head towards the north. Sex: archaeology – M, anthropology – ?, aDNA – M. Age: adultus – maturus (20–60). Grave goods: no finds. Archaeological dating: Bell Beaker culture, late stage. Radiocarbon dating: not available *(193).* Pandora No.: VLI030. NM Prague Inv. No.: P7A 41577.

44.4. Vliněves – Early Bronze Age

Grave 40 (Group 1): Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – F?, aDNA – F. Age: adultus I (20–25). Grave goods: no. Archaeological dating: Early Únětice culture (based on the position in the grave group). Radiocarbon dating: not available *(116).* Pandora No.: VLI047. NM Prague Inv. No.: P7A 40930.

Grave 75 (Group 1): Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: adult (over 20). Grave goods: one small vessel, bronze pin of the Cyprus type, five bronze earrings, necklace made of bronze spirals and amber beads. Archaeological dating: Classic Únětice culture (BzA2). Radiocarbon dating: UBA-23635

(3536±34) 1953–1754 cal BC 2-sigma; CRL-19336 (3554±33) 2014–1772 cal BC 2-sigma *(116).* Pandora No.: VLI046. NM Prague Inv. No.: P7A 40976.

Grave 361 (Group 6): Skeleton: right-sided crouched burial, head towards the south-south-west. Sex: anthropology – ?, aDNA – no aDNA. Age: adult (over 20). Grave goods: two vessels. Archaeological dating: Early Únětice culture, cemetery phase 4+5. Radiocarbon dating: MAMS-30769 (sample is missing) *(116).* Pandora No.: VLI063. NM Prague Inv. No.: P7A 41740.

Grave 371 (Group 8): Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – child, aDNA – F. Age: infans (to 7). Grave goods: no. Archaeological dating: Early (?) Únětice culture, based on position towards grave no. 370). Radiocarbon dating: not available *(116).* Pandora No.: VLI039. NM Prague Inv. No.: P7A 41095.

Grave 395 (Group 9): Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – F, aDNA – F. Age: adultus (20–40). Grave goods: one small vessel, two bronze pins of the Únětice type, two bronze earrings, one bronze lock-ring, 21 bronze beads and five amber beads. Archaeological dating: Classic Únětice culture, cemetery phase 5. Radiocarbon dating: UBA-27395 (3623±58) 2193–1780 cal BC 2-sigma *(116).* Pandora No.: VLI045. NM Prague Inv. No.: P7A 41650.

Grave 437 (Group 9):

Skeleton 1: right-sided crouched burial, head towards the south-south-west. Sex: anthropology – F?, aDNA – F. Age: adultus (20–40). Grave goods: one vessel, two bronze pins of Únětice type, two bronze earrings. Archaeological dating: Classic Únětice culture. Radiocarbon dating: older than 14C-dated primary burial skeleton 2 (see below) *(116).* Pandora No.: VLI060. NM Prague Inv. No.: P7A 41777.

Skeleton 2: right-sided crouched burial, head towards the north-east. Sex: anthropology – ?, aDNA – F. Age: maturus (over 40). Grave goods: no. Archaeological dating: Classic Únětice culture. Radiocarbon dating: UBA-27396 (3618±52) 2139–1824 cal BC 2-sigma *(116).* Pandora No.: VLI065. NM Prague Inv. No.: P7A 41662.

Grave 443 (Group 9): Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – child, aDNA – M. Age: infans (7–9). Grave goods: bronze pin of Únětice type, bronze bracelet, amber bead/pendant. Archaeological dating: Classic Únětice culture (BzA2). Radiocarbon dating: UBA-27398 (3504±41) 1936–1698 cal BC 2-sigma *(116).* Pandora No.: VLI042. NM Prague Inv. No.: P7A 41671.

Grave 459 (Group 9):

Skeleton 2: right-sided crouched burial, head towards the south. Sex: anthropology – F?, aDNA – no aDNA. Age: adultus II – senilis (over 30). Grave goods: bronze pin of Únětice type, fragments of three to four bronze earrings, necklace made of bronze spirals and amber beads. Archaeological dating: Classic Únětice culture (BzA2). Radiocarbon dating: not available *(116).* Pandora No.: VLI041. NM Prague Inv. No.: P7A 41665.

Grave 465 (Group 9): Skeleton: right-sided crouched burial, head towards the south. Sex: anthropology – M?, aDNA – no aDNA. Age: adultus (20–40). Grave goods: no. Archaeological dating: Classical Únětice culture (?) (based on position in the grave group 9). Radiocarbon dating: not available *(116).* Pandora No.: VLI0040. NM Prague Inv. No.: P7A 41672.

Grave 504 (Group 12): Skeleton: right-sided crouched burial, head towards the north. Sex: anthropology – F, aDNA – F. Age: maturus II – senilis (over 50). Grave goods: two bronze nail-headed pins. Archaeological dating: early beginning of the Middle Bronze Age Tumulus culture (BzA2/B1–BzB1). Radiocarbon dating: not available *(116).* Pandora No.: VLI053. NM Prague Inv. No.: P7A 41397.

Grave 514 (Group 12):

Skeleton 1: bones disturbed and dislocated, position of the skeleton indeterminable. Sex: anthropology – M?, aDNA – M. Age: maturus II – senilis (over 50). Grave goods: fragmented bronze pin of Únětice type. Archaeological dating: Classic Únětice culture (BzA2). Radiocarbon dating: UBA-23640 (3509±29) 1913–1749 cal BC 2-sigma *(116).* Pandora No.: VLI054. NM Prague Inv. No.: P7A 41440.

Grave 515 (Group 13): Skeleton: right-sided crouched burial, head towards the south-east. Sex: anthropology – F, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: one small vessel. Archaeological dating: Únětice culture, cemetery phase 4+5+6. Radiocarbon dating: no (MAMS-30767, insufficient collagen) *(116).* Pandora No.: VLI062. NM Prague Inv. No.: P7A 41725.

Grave 517 (Group 13): Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – F, aDNA – F. Age: maturus (over 40). Grave goods: two vessels, three animal teeth. Archaeological dating: Proto-Únětice/Early Únětice culture, cemetery phase 2. Radiocarbon dating: not available *(116).* Pandora No.: VLI061. NM Prague Inv. No.: P7A 41726.

Grave 520 (Group 13): Skeleton: left-sided crouched burial, head towards the south-west. Sex: anthropology – ?, aDNA – F. Age: maturus – senilis (over 40). Grave goods: two vessels. Archaeological dating: Early Únětice culture, cemetery phase 3/4. Radiocarbon dating: UBA-23642 (3725±28) 2200–2035 cal BC 2-sigma *(116).* Pandora No.: VLI058. NM Prague Inv. No.: P7A 41735.

Grave 521 (Group 13): Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – F?, aDNA – F. Age: juvenis – adultus I (15–25). Grave goods: two vessels. Archaeological dating: Early Únětice culture, cemetery phase 2. Radiocarbon dating: MAMS-30768 (3677±25) 2139–1977 cal BC 2-sigma *(116).* Pandora No.: VLI064. NM Prague Inv. No.: P7A 41724.

Grave 533 (isolated grave): Skeleton: right-sided crouched burial, head towards the west-west-south. Sex: anthropology – M?, aDNA – no aDNA. Age: adultus – maturus (20–60). Grave goods: bronze dagger, bronze axe, bone awl. Archaeological dating: Classic/Post-Classic (Late) Únětice culture (BzA2–BzA2/B1). Radiocarbon dating: UBA-23643 (3454±27) 1878–1691 cal BC 2-sigma; CRL-19344 (3492±21) 1884–1750 cal BC 2-sigma *(116).* Pandora No.: VLI048. NM Prague Inv. No.: P7A 41742.

Feature 2415 (settlement pit): Skeleton: right-sided crouched burial, head towards the south-west. Sex: anthropology – M, aDNA – no aDNA. Age: maturus – senilis (over 40). Grave goods: flint dagger. Archaeological dating: BzA2–BzA2/BzB1. Radiocarbon dating: MAMS-30770 (3454±24) 1877–1692 cal BC 2-sigma *(116).* Pandora No.: VLI0049. NM Prague Inv. No.: P7A 41084.

Feature 2427 (settlement pit with human skeleton): Skeleton: left-sided crouched burial, head towards the south. Sex: anthropology – F, aDNA – F. Age: maturus (40–60). Grave goods: one vessel, two fragments of grinding stones. Archaeological dating: Classic to Post-Classic Únětice culture, cemetery phase 6. Radiocarbon dating: UBA-23638 (3526±36) 1947–1749 cal BC 2-sigma; + from animal bone (sheep/goat neonat. UBA-23646 (3348±27) 1735–1534 cal BC 2-sigma *(116).* Pandora No.: VLI050. NM Prague Inv. No.: P7A 41085.

Feature 2544 (settlement pit): Skeleton: left-sided (?) crouched burial, head towards the south-west, dislocated parts of skeleton. Sex: anthropology – M, aDNA – M. Age: maturus II – senilis (over 50). Grave goods: Without directly related goods (only settlement discard from the fill of feature). Archaeological dating: Classic to Post-Classic Únětice culture. Radiocarbon dating: UBA-23639 (3378±30) 1745–1615 cal BC 2-sigma; MAMS-44712 (3449±25) 1877-1689 cal BC 2-sigma *(116).* Pandora No.: VLI051. NM Prague Inv. No.: P7A 41088.

45. Zeleneč (Zeleneč, Prague-East district, central Bohemia, Czech Republic)

Contact person: Miroslav Dobeš

Rescue excavation prior to the construction of a group of family homes in 2004 (J. Špaček). The excavation area provided more than one-hundred graves from the Early Middle Ages also four graves from the Corded Ware culture, from which one skeleton described below was analysed for aDNA. The Corded Ware culture graves dated to its late phase were published in full in *(195).*

Grave 18. Skeleton: left-sided crouched burial, head towards the east. In the grave together with a right-sided crouched burial, head towards the east (infans, around 1). Sex: archaeology – F, anthropology – ?, aDNA – F. Age: juvenis (15–20). Grave goods: two amphorae, beaker, pot, single handled cup, two handled cup, another vessel, stone battle axe, copper spiral temple ring, copper wire, whetstone, chipped industry – blade. Archaeological dating: Corded Ware culture, local (late) stage. Radiocarbon dating: not available *(195).* Pandora No.: ZEL001. NM Prague Inv. No.: P7A 18774.

Supplementary Figures

Fig. S6. Ternary plot showing the percentage of ancestry of distal three-way models. Using *qpAdm* each ancient Bohemian individual is modelled as a mixture of ancestry sources ascribable to Anatolia_Neolithic and WHG (for individuals without "steppe" ancestry) and Anatolia_Neolithic, WHG and Yamnaya_Samara (for individuals with "steppe" ancestry). Shapes indicate sex of individual and colors indicate degree of model fits. List of outgroups and ancestry percentages are found in Table S9.

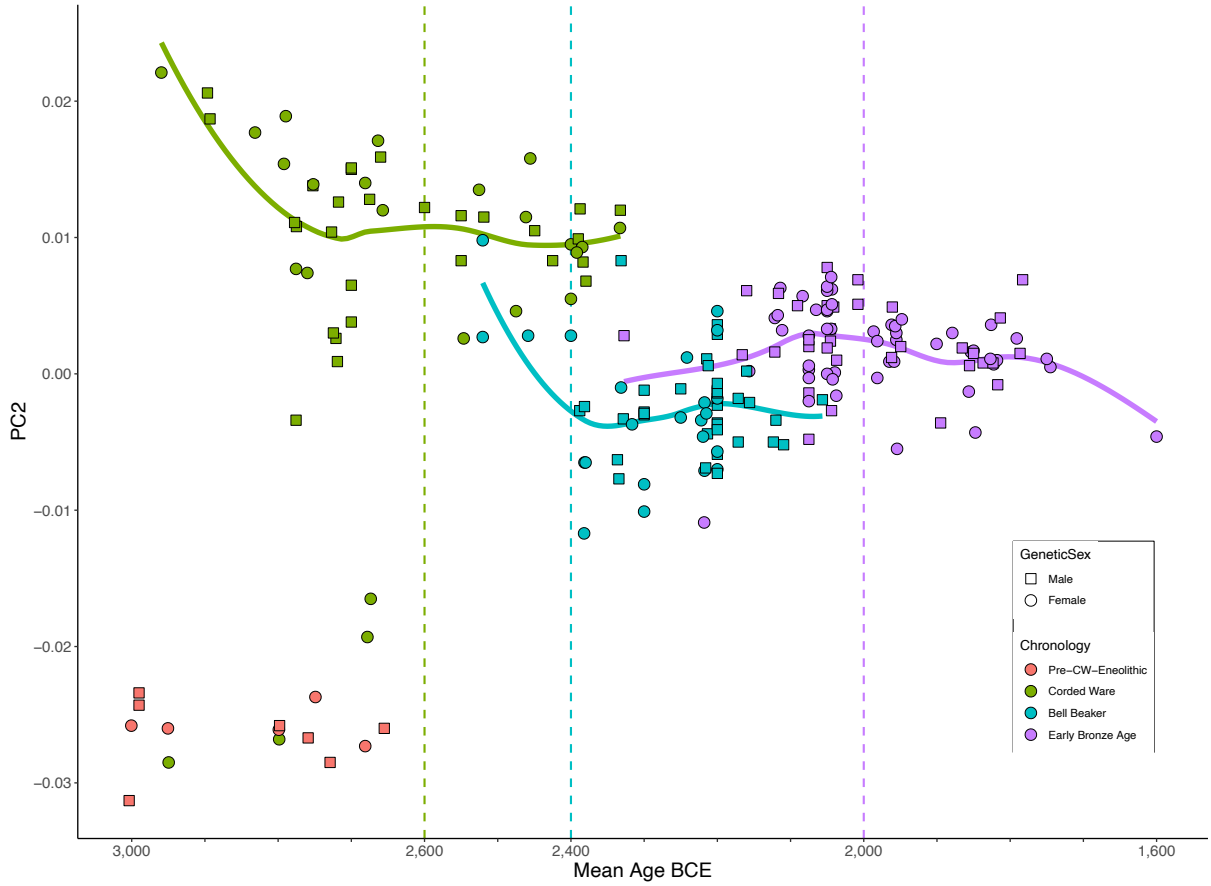

Fig. S7. Temporal variation in PC2 with loess regression curves. Colors indicate culture, and dashed vertical lines indicate grouping of cultures into early/late phases.

Table S1-S36. (separate Excel spreadsheet). Supplementary tables are too large and have been burnt onto CD which accompanies this thesis.

Table S1: Chronology of archaeological cultures in Bohemia.

Table S2: Geographic and chronological information of published and newly reported sites.

Table S3: Contextual information, sequencing statistics and basic genetic results for all newly analyzed samples.

Table S4: Contextual and genetic overview of quality filtered ancient individuals from Bohemia analysed in this study.

Table S5: Contextual information for previously published samples used in analyses which appear in this publication.

Table S6: Summary table of newly reported (n=140) and previously published 14C dates.

Table S7: List of 1,141 modern West Eurasian individuals on which Principal Components Analysis was conducted.

Table S8: qpAdm modelling of each pre-CW individual from Bohemia as a two-way mixture of Anatolia_Neolithic and a hunter-gatherer source.

Table S9: qpAdm modelling of ancient Bohemians as either three-way mixtures of Anatolia_Neolithic, WHG and Yamnaya_Samara (individuals with steppe ancestry) or Anatolia_Neolithic and WHG (individuals without steppe ancestry i.e. pre-CW Eneolithic and Bohemia_CW_noSteppe).

Table S10: qpAdm modelling of each pre-CW cultural group in Bohemia as a three-way mixture of Anatolia_Neolithic, Loschbour and Koros_HG.

Table S11: DATES estimate of when hunter-gatherer ancestry was intogressed into each pre-CW cultural group in Bohemia.

Table S12: qpAdm modelling of Bohemia_PE (Jordanow) and Boemia_EE (Funnelbeaker) as mixtures of Anatolia_Neolithic and different hunter-gatherer sources.

Table S13: Testing cladality between Bohemia_PE and Bohemia_ME using qpWave.

Table S14: qpAdm modelling of Bohemia_ME_Rivnac and Bohemia_ME_GAC (Globular Amphora Culture) as mixtures of Anatolia_Neolithic and different hunter-gatherer sources.

Table S15: Modelling Bohemia_CW_Early as a two-way mixture using proximal sources.

Table S16: qpAdm modelling Bohemia_CW_Early as a three-way mixture using distal sources.

Table S17: Modelling Bohemia_CW_Early using proximal sources.

Table S18: Modelling Bohemia_CW_Late using proximal sources.

Table S19: Modelling Germany_Corded_Ware using proximal sources.

Table S20: f4 statistics in the form of f4(Mbuti, CordedWare; Yamnaya, ancient northeast Europe).

Table S21: f4 statistics in the form of f4(Mbuti, ancient northeast Europe; Yamnaya, CordedWare).

Table S22: f4-statistics in the form of f4(W, X; Y, Z) showing that VLI009 and VLI079 carry significantly more hunter-gatherer ancestry than Bohemia_ME groups.

Table S23: f4-statistics in the form f4(W, X; Y, Z) showing that Bohemia_CW_Late has significantly more Middle Eneolithic-like ancestry compared to Bohemia_CW_Early (without the earlyCW with no "steppe" ancestry).

Table S24: f4-statistics in the form f4(W, X; Y, Z) showing that Bohema_CW_Late has equal amount of Middle Eneolithic-like ancestry as to Bohemia_CW_Early (when Bohemia_CW_Early includes the early CW females without "steppe" ancestry).

Table S25: qpAdm modelling of Bohemia_CW_Late as mixtures of Bohemia_CW_Early and a local pre-CW source.

Table S26: qpWave modelling of Bohemia_CW_Late as being cladal with Bohemia_CW_Early (including individuals without "steppe" ancestry).

Table S27: Pairwise Fst values between 3 highest and 3 lowest early CW on PC2 and between modern European populations.

Table S28: qpAdm modelling of Bohemia_BB_Early using proximal sources.

Table S29: f4-statistics in the form of f4(W, X; Y, Z) showing that Bohemia_BB_Late as significantly more Middle Eneolithic ancestry compared to Bohemia_BB_Early.

Table S30: qpAdm modelling of Bohemia_BB_Late as a two-way mixture of Bohemia_BB_Early and a local Middle Eneolithic source.

Table S31: f4-statistics in the form of f4(W, X; Y, Z) showing that Bohemia_Unetice_preClassical carries significantly more non-local ancestry compared to Bohemia_BB_Late.

Table S32: Admixture f3 statistics in the form of f3(A, B; C).

Table S33: qpAdm modelling of Bohemia_Unetice_preClassical using proximal sources.

Table S34: f4-statistics in the form of f4(W, X; Y, Z) showing that VLI051 is a significant outlier compared to all Bohemian cultural groups.

Table S35: Testing cladality between Bohemia_Unetice_preClassical and Bohemia_Unetice_Classical using qpWave.

Table S36: f4-statistic in the form of f4(W, X; Y, Z) showing that Bohemia_Unetice_Classical carries significantly less EHG and Yamnaya-like ancestry and more Neolithic-like ancestry.

Table S37: qpAdm modelling of Bohemia_Unetice_Classical as a two-way mixture of Bohemia_Unetice_preClassical and a local Middle Eneolithic source.

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5. Manuscript B

An Early Bronze Age community on the Amber Road – kinship and social behaviour in Mikulovice, Eastern Bohemia.

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Abstract
The Early Bronze Age in Europe is characterised by continent-wide interconnectedness, manifested through long-distance exchange of goods and increased individual mobility. However, little is known about how this worked and impacted communities at the local scale, their social organisation, what proportion and subset of the society was mobile and their pattern of mobility. In order to elucidate these questions, we analysed genetic data from 92 individuals buried at an Únětice cemetery in Mikulovice, located on the Amber road and known for its rich and exotic grave goods. We find that 81/92 individuals have close relatives at Mikulovice, forming 12 biological kinship groups, spanning up to 4 generations. Social behaviour appears to have been diverse, ranging from the same couple having six children to individuals having offspring with multiple partners. More broadly, individuals from Mikulovice constituted a genetically homogeneous population with evidence of higher mobility of females compared to males. Several stable isotope outliers have parents and/or grandparents buried at Mikulovice, suggesting a pattern of childhood mobility between birth and death in Mikulovice.

Introduction

Recent studies on the Early Bronze Age (EBA) of Europe have revealed a highly interconnected, mobile, and globalised world with "movement, travelling, trafficking, encounters and interaction as absolutely crucial to the creation of human life-worlds" (Vandkilde et al. 2015). The "enormous expansion of long-distance travel and exchange" (Kristiansen and Larsson 2005) led to a "truly international network of metal trade and exchange" which resulted in distant regions becoming "dependant on each other" (Kristiansen and Larsson 2005, Kristiansen 1998). Essential materials such as "copper and tin, or bronze in finished or semi-finished form, had to be distributed to all societies throughout the known world from a few source areas" (Kristiansen 2017) driving "an economic need to maintain open lines of long-distance exchange in order to secure the distribution of metal" (Kristiansen and Larsson 2005). Consequently, "migrations, travels and other forms of interaction and mobility have come to the forefront of archaeological interpretation and debate" (Kristiansen 2017) when discussing EBA Europe, as can be seen in many recent publications (Kristiansen and Larsson 2005, Harding 2013, Pokutta 2013, Suchowska-Ducke et al. 2015a, Suchowska-Ducke et al. 2015b, Vandkilde 2016, Kienlin 2017, Kristiansen et al. 2018, Meller 2019, Ernée et al. 2020).

One key question, and perhaps a less understood aspect of the large-scale, continent-wide interconnectivity of EBA Europe, is "how an interconnected Bronze Age world impacted local communities" (Vandkilde et al. 2015) and/or what parts of the EBA communities were indeed actively involved in such supra-regional long-distance networks of contacts, exchange and trade (Ernée et al 2020). To better understand these dynamics at a local scale, detailed investigations of "micro-scale studies are extremely useful" (Vandkilde et al. 2015, Mittnik et al. 2019, Furholt 2019a, Furholt 2019b). With the detailed archaeogenetic investigations of the EBA cemetery in Mikulovice (Czech Republic) presented in this study, we try to address the aforementioned gaps in our understanding.

Bohemia has been densely settled since the beginning of the Neolithic (Pavlů & Zápotocká 2013) and has "benefited from the excellent geographic location" (Ernée et al. 2009), located in the heart of Europe and on the crossroads of important long-distance communication and exchange networks, where many supraregional cultural phenomena (including Corded Ware and Bell Beakers) have interacted (Papac et al. 2021, manuscript A). The long archaeological research tradition in Bohemia has facilitated hundreds of EBA cemeteries with thousands of inhumation graves to be available for detailed archaeogenetic analyses, with many of them having their archaeological contexts recently published (Ernée 2015, Limburský et al. 2018, Ernée et al. 2020).

The EBA cemetery in Mikulovice under focus here is associated with the Únětice culture (UC), named after the eponymous site in Únětice near Prague, Czech Republic (Fig. 1A) (Ryzner 1880a, Ryzner 1880b). Sharing mostly very similar burial practices (both sexes in right-side crouched position, head pointing south, facing east), settlement structure (e.g. forms of houses and storage pits) and types of artefacts (typical Únětice cups, bronze eyelet pins, bronze hoards), UC-associated regional groups have been identified from central Germany to southwestern Slovakia, and Silesia to lower Austria. UC-associated elements date from 2300/2200 to 1750/1700 BCE, and this range is typically divided into an older (^{14}C ~2300/2200–2000/1950 BCE) and younger (classical/post-classical) (^{14}C ~2000/1950–

1750/1700 BCE) phase (Moucha 1961, Moucha 1963, Bartelheim 1998, Zich 1996, Jiráň et al 2013, Bátora 2018; Ernée et al. 2020).

The entire Únětice domain, along with the Wessex/Atlantic, Danubian and Argaric regions, belongs to an important area of the non-Mediterranean European EBA world, straddling a key geographic position and influencing the transfer of copper/bronze from the eastern Alpine source areas to northern continental Europe and Scandinavia during the Nordic Late Neolithic II and beginning of the Bronze Age (~2000-1700 BCE) (Vandkilde 2017; Nørgaard et al. 2019). The Bohemian Únětice group, as represented by its large cemeteries with hundreds of well-equipped inhumations rich in amber, gold, bronze, and other "exotics" (imported artefacts, raw materials, and/or technologies of a demonstrably foreign origin) was one of the most important hubs of the Únětice world (Rassmann 1996, Zich 1996, Bartelheim 1998, Müller et al. 1999, Müller 1999, Krause 2003, Moucha 2005, Ernée et al. 2020).

In this period of "cross-cultural movements of commodities in central Europe" (Fischl and Kiss 2015) when "Slovakian and Alpine copper ores and Baltic amber were imported into central Europe" (Fischl and Kiss 2015, Ernée et al. 2009, Ernée 2015), Mikulovice was strategically situated directly on the important long-distance route, which later developed into the so-called Amber Road (Ernée 2012, Ernée 2013, Ernée 2016, Ernée 2017). The Únětice cemetery in Mikulovice is well known for its extraordinary amount and quality of grave goods. Aside from many "exotics", this also includes amber artefacts, simple beads, and multiple bored spacers of diverse forms used as parts of rich necklaces, which were deposited in female graves dated exclusively to the classical UC (~2000-1800/1750 BCE). With 882 amber artefacts identified in 27 graves (28% of all graves), Mikulovice contains the richest assemblage of amber in the EBA world. Within the largest grave group, group A, amber was found in 45.5% of female graves, and in 40.6% of females graves in the entire cemetery (Fig. 1B). The richest grave in Mikulovice (MIB009 - grave 2) contains the remains of a 35-40-year-old female along with two golden earrings, three bronze eyelet pins, five bronze bracelets, several sea shells and a necklace composed of at least 416 amber beads, and also happens to be the best equipped amber grave ever found in EBA Europe (Ernée 2012, Ernée 2013, Ernée 2016, Ernée 2017; Ernée et al. 2020).

Stable isotope analyses of EBA Mikulovice individuals have revealed a group comprising of predominantly locals, with less than 10% of the population not being born in close vicinity of the cemetery, thus identified as non-local. This suggests that only a limited part of community, possibly the local "authorities" or "elites" in the broadest sense, may have been involved in the act of moving goods and exotics along the long-distance routes and networks of supra-regional contacts and exchange (Ernée et al. 2020).

In contrast to the sex/gender differentiation in body position of the preceding Late Eneolithic Corded Ware (CW) and Bell Beaker (BB) mortuary traditions, Únětice graves show greater sex/gender differentiation in terms of grave goods, with unambiguously greater "luxury" in female compared to male graves. This is represented mainly in personal jewellery items, such as various types of bronze or gold adornments, necklaces (often composed of dozens or hundreds of amber beads), bronze pins, arm rings, golden earrings, and others. This pattern of greater luxury in female graves is a practice broadly shared with

many adjacent central European EBA groups, including Danubian regions associated with the Straubing and Unterwölbling cultural groups (Shennan 1975, Massy 2018, Ernée et al. 2020), but interestingly not with other parts of the Únětice world (e.g. Central Germany, Silesia, lower Austria).

The last few years have seen great progress in the analysis of the EBA cemetery in Mikulovice, resulting in a broad range of archaeological, anthropological, isotopic and other scientific results (Ernée et al. 2020). Such a complex multidisciplinary analysis, so far rare in research into European prehistory (Stockhammer et al. 2015a, Stockhammer et al. 2015b, Knipper et al. 2017, Massy et al. 2017, Massy 2018, Mittnik et al. 2019), lends a unique and detailed insight into a local EBA population. Here we present and contextualise an archaeogenetic investigation of the Mikulovice EBA cemetery.

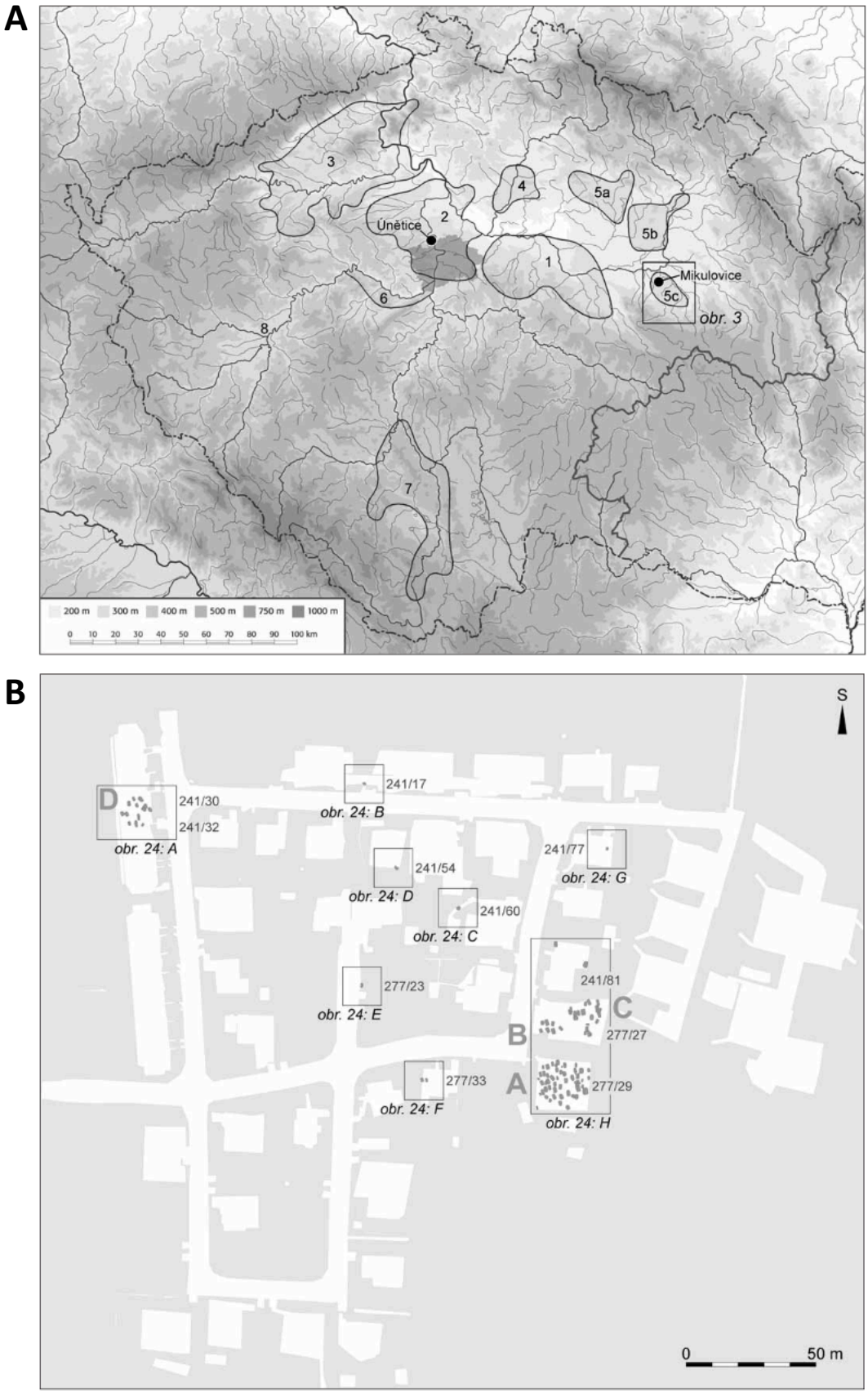


Fig. 1. Geographic context of the Mikulovice cemetery. Figure adapted from Ernée et al. 2020. (A) Map of Bohemia showing the location of Mikulovice along with the 7 regional Bohemian Únětice groups (Moucha 1961, Moucha 1963). (B) Site plan of Mikulovice rescue excavations showing the spatial distribution of graves and grave groups (A, B, C, D).

Results

The EBA cemetery in Mikulovice contains 101 burials (98 graves and 3 pits) with the remains of 109 individuals. A total of 101 individuals with sufficient macroscopic preservation were sampled for ancient DNA analysis, making Mikulovice one of the largest and best sampled prehistoric cemeteries so far. After screening for ancient DNA preservation, filtering out samples with poor DNA preservation and/or contamination, 92 (46 male and 46 female) individuals were chosen for downstream analysis (Table S1, Table S2, Table S3), of which fifteen have been reported previously (Papac et al. 2021, manuscript A).

Population genetic analyses

The uniparentally inherited (mitochondrial and Y-chromosome) lineages found among the EBA individuals buried at Mikulovice are typical of west Eurasian populations. The dominant Y-chromosome lineage is R1b-P312 (n=36), with all individuals who have coverage at the downstream L2/S139 also showing the derived allele (Table S1). R1b-P312 is first attested in Bohemia in the BB period (100%), after which it drops to ~20% in the pre-classical Bohemian Únětice phase (Papac et al. 2021, manuscript A). The next most frequent (n=3) haplogroup is R1a-Z645 (and derived lineages e.g. Z647 and Z651). This lineage is absent from central European CW, and is instead found at high frequency in Baltic and Scandinavian CW (Saag et al. 2017, Malmström et al. 2019), appearing in central Europe for the first time in the EBA (Papac et al. 2021, manuscript A). R1b-L151 (n=1) is the most common lineage in early CW from Bohemia, but goes unsampled thereafter until this individual at Mikulovice (Papac et al. 2021, manuscript A). I2a2a (n=1) and G2a2b2a (n=1) are commonly found in Neolithic communities across Europe (Mathieson et al. 2018, Schröder et al. 2019).

Mitochondrial haplogroup diversity is higher (34 different haplogroups) and more evenly apportioned, with the most frequent haplogroups (J1c and K1b) represented 7 times each (7/92, 7.6%). This is in stark contrast to the Y lineage diversity, which is dominated (>85%) by a single lineage (R1b-P312). Although similar discrepancies in uniparental frequencies are often interpreted as the result of patrilocality and/or female exogamy (Kayser et al. 2003, Nasidze et al. 2004), the observed pattern could be distorted by the decline in Y-chromosome diversity of the Neolithic and Eneolithic (Karmin et al. 2015, Zeng et al. 2018), whereby even an influx of males from neighbouring regions would not dramatically increase intra-site Y-chromosomal diversity due to low background diversity. Detailed analyses of biological kinship and patterns of inheritance at Mikulovice will shed more light on rates of patri- vs matrilocality.

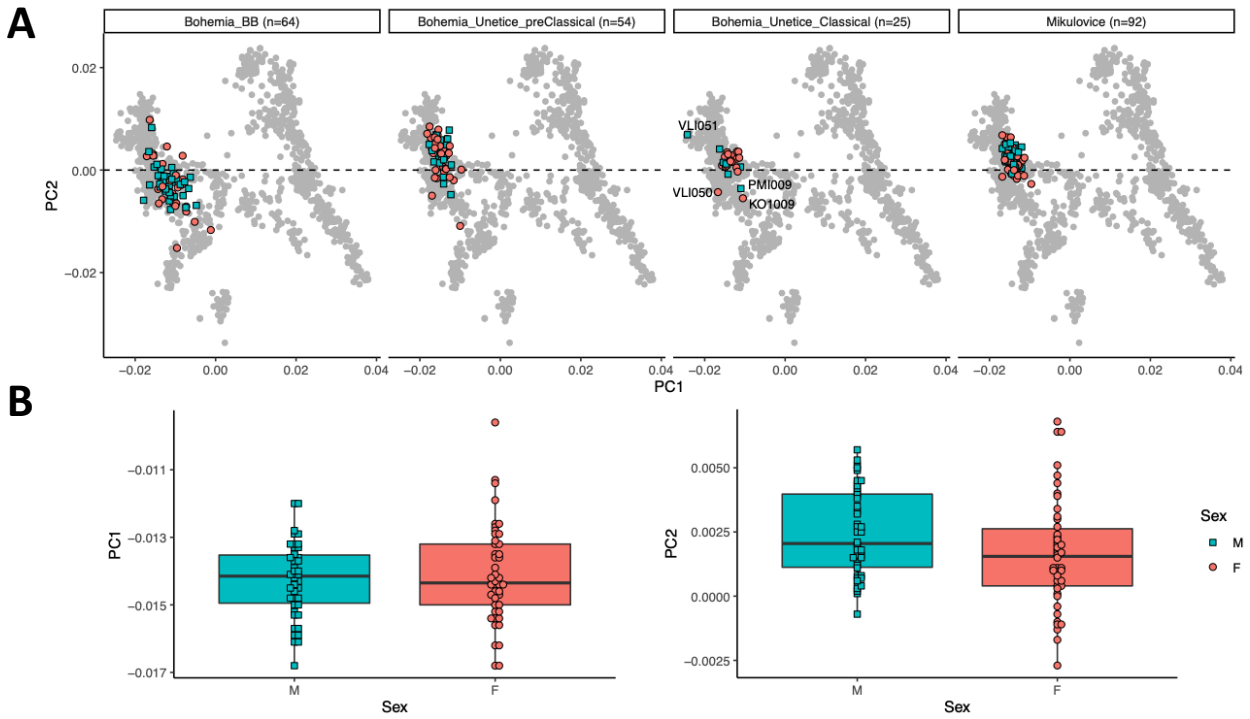


Fig. 2. Genetic diversity of Mikulovice population. (A) Principal components analysis (PCA) constructed on 1,141 modern west Eurasian individuals (Table S4) with ancient individuals projected showing the genetic variation of Mikulovice in the context of preceding and contemporaneous individuals from Bohemia (Bell Beaker, Preclassical Únětice, Classical Únětice). (B) Genetic PC1 and PC2 variation of the Mikulovice EBA population broken down by sex showing larger variation in females.

From an autosomal perspective, the population buried at Mikulovice largely resembles previously studied EBA populations of Europe. We find that the Mikulovice EBA individuals occupy similar positions in PCA (Fig. 2) space as previously studied CW, BB and EBA individuals. Using *qpAdm*, it is possible to model ($p>0.05$) most (83/92) individuals as being three-way mixtures of Loschbour (western hunter-gatherer), Anatolia_Neolithic and Yamnaya_Samara (Table S5), with the majority carrying 50-65% “steppe”-related ancestry. This is intermediate to the amount of “steppe”-related ancestry found in central European Corded Ware (>65%) and Bell Beaker (<50%) individuals (Papac et al. 2021, manuscript A).

We find that the Mikulovice EBA population is generally more closely related to other Bohemian classical Únětice groups than it is to preclassical Únětice groups. This can be seen in *qpWave* cladality tests where the whole Mikulovice EBA population, certain subsets thereof (Table S6), or individuals (Table S7), are generally better modelled as being cladal with Bohemian Classical Únětice than with Bohemian preclassical Únětice.

Biological and social kinship

We used READ (Monroy Kuhn et al., 2018), lcmlkin (Lipatov et al., 2015) and genotype mismatch rates between pairs of individuals to infer biological relatedness of the Mikulovice EBA population (Table S3). In doing so, we were able to reconstruct pedigrees of biological relationships and descent. Of the 92 individuals analysed, 81 had close relatives (closer than

5th degree related) at the site and belonged to one of 12 biological kinship groups (or tentatively, “families”), ranging in size from 2 to 13 individuals (Fig. 3, Supplementary Information). Eleven individuals showed no close relationship to anyone else buried at Mikulovice.

Spatial distribution of graves and biological kinship groups

The Mikulovice EBA cemetery is made up of four main clusters of graves, named A, B, C, D, along with 11 graves/burials dispersed throughout the cemetery and spatially not part of these four main clusters (Fig. 1B).

We find that the biological kinship groups tend to be buried in close proximity, suggesting a non-random distribution of graves. This is best seen in biological kinships groups 1, 2, 3, 9, 10, and 11 (Supplementary Information). Individuals with no close biological relatives (n=11) are evenly dispersed throughout the cemetery, with 3 buried in group A, 3 in group B, 1 in group C, and 4 buried in graves not associated with any of the 4 major grave clusters.

The majority of burials contain the remains of a single individual. There are only four double (graves 11, 64, 81, and 92) and two triple burials (graves 28 and 97). In both triple burials first degree relatives are present. Grave 28 contains the remains of a father (MIB043) and his two children (MIB041 and MIB042), whilst grave 97 contains the remains of a mother (MIG010) and son (MIG008), along with a second degree relative of the son (MIG009, possibly paternal grandfather, paternal uncle, or half-brother). Among the double burials which yielded ancient DNA data, two contained close relatives (father and daughter in grave 11, half-brothers in grave 92). The senile male (MIB006) individual and the female (MIB005, aged 50+) in grave 64 are neither related to each other, nor do they have relatives within the cemetery Their co-burial may reflect a social kinship, possibly a couple who had offspring which were not buried at Mikulovice. It appears as though multiple graves at Mikulovice were reserved for kin, however without a clear or specific pattern of kinship, with father-offspring, mother-offspring, siblings, half-siblings, and unrelated individuals found together.

The biological relationships found between the single clusters of graves are important for the understanding whether the cemetery was used by the same community or by different groups of individuals and for how long it was in use. We find biologically related individuals buried in different grave clusters, although not closer than 3rd degree related. Kinship group 6, for example, consists of individuals found widely distributed across the cemetery, including in grave clusters A, C and D, as well as two isolated burials, suggesting the entire cemetery was used by the same community.

Biological kinship groups

The reconstruction of biological kinship groups reveals a total of twelve groups, ranging from two to thirteen individuals in each group (Supplementary Information, Fig. 3). Eleven individuals do not have close (closer than 5th degree) biological kin buried at Mikulovice. The majority of individuals without close relatives are female (8/11) and adults (10/11), with the only juvenile being an 8-9-year-old girl (MIB007 – grave 53). Such a pattern of adult females being the most common demographic group among individuals without biological kin at Mikulovice is consistent with higher female mobility during EBA central Europe.

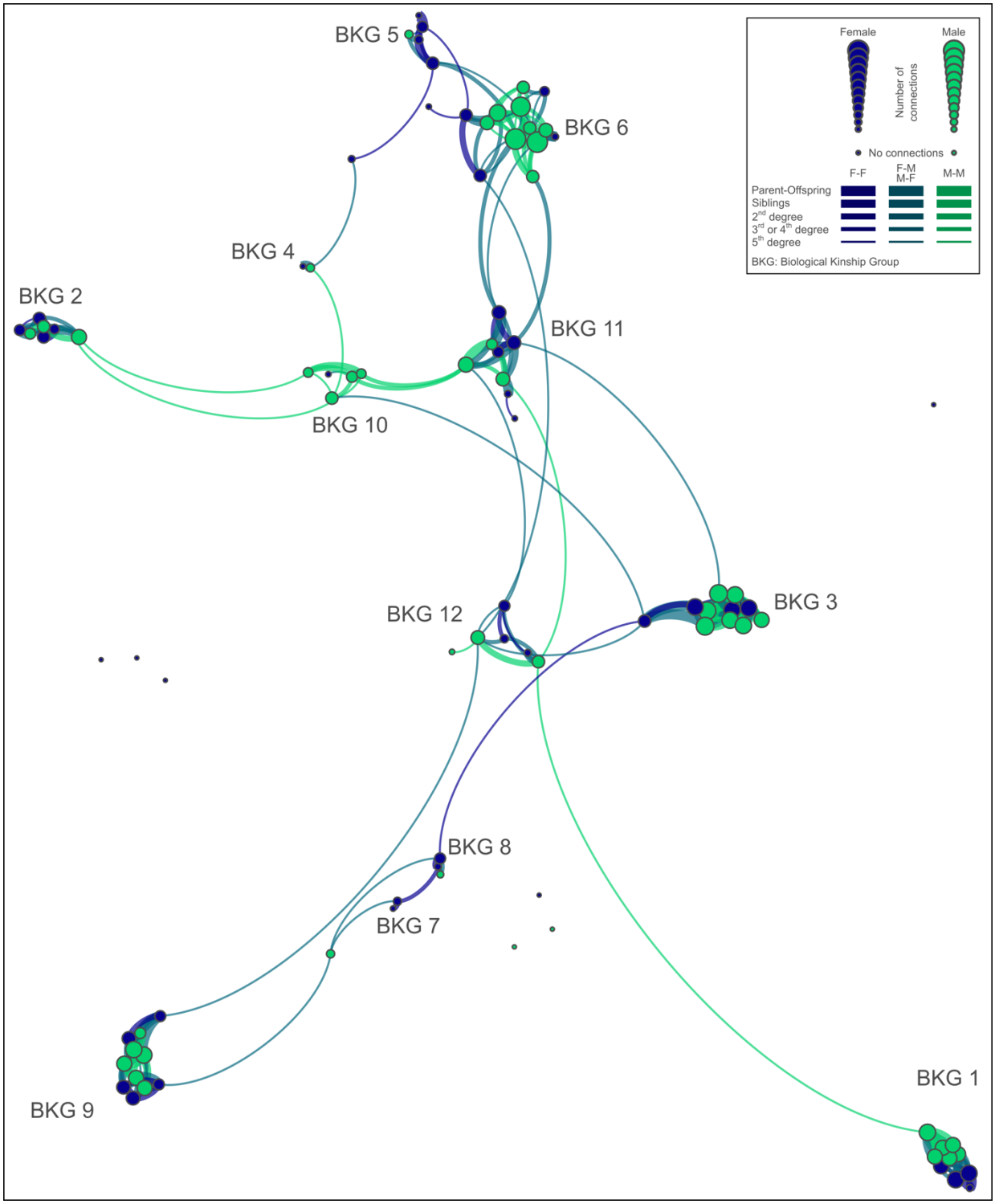


Fig. 3. Network summary of Biological Kinship Groups (BKG) at Mikulovice. The relationships between BKGs are visualised in a network. Each individual is a point with the size of the point related to how many relatives they have at Mikulovice. Colour of the point indicates biological sex and lines show biological connections between BKGs. Thickness of line is related to degree of relationship.

The kinship tree (pedigree) with the most number of generations comes from biological kinship group 3. This is the only tree for which four generations are required to fit the data. Biological kinship groups 2, 5, 9, and 11 require at least three generations. Such a pattern of relatively shallow kinship trees (3-4 generations), along with biological relationships between grave clusters in the cemetery (A, B, C, D, Fig. 1B), is consistent with grave clusters being used approximately contemporaneously and the majority of the cemetery being used for less than 150 years.

Surprisingly, the reconstructed kinship trees show an overrepresentation of subadults in their final generation. This is the case for twelve out of the thirteen subadults (<20 years of age) in the kinship trees with three generations or more (kinship group 2, 3, 9, 11). MIB003 (grave 68) of kinship group 3 is the only exception. It is not clear whether such a pattern is the result of changing mortuary behaviour at the end of the cemetery's use, in which children were more readily given an inhumation burial, or an increased child mortality rate towards the end of the cemetery's use.

In general, our reconstructed kinship trees contain a large number of inferred individuals who have not been identified as being buried at the cemetery (n=~50), suggesting a large portion of the community was either buried elsewhere or not given an inhumation burial. This includes several cases of young children with unidentified parents (e.g. MIB020 – grave 39, MIB040 – grave 25, MIB046 – grave 31, MIB054 – grave 42, MIB058 – grave 48, MIB070 – grave 67). Biological kinship group 9 has three children aged 4-7 whose parents have not been identified. In only three instances do we find both parents present along with their child(ren). In cases where only one parent has been identified, the father (n=11) is present slightly more frequently than the mother (n=9).

By analysing the average relatedness of each individual in the community, we find a general higher relatedness of males than females (Fig. 4). This is reinforced by the number of first

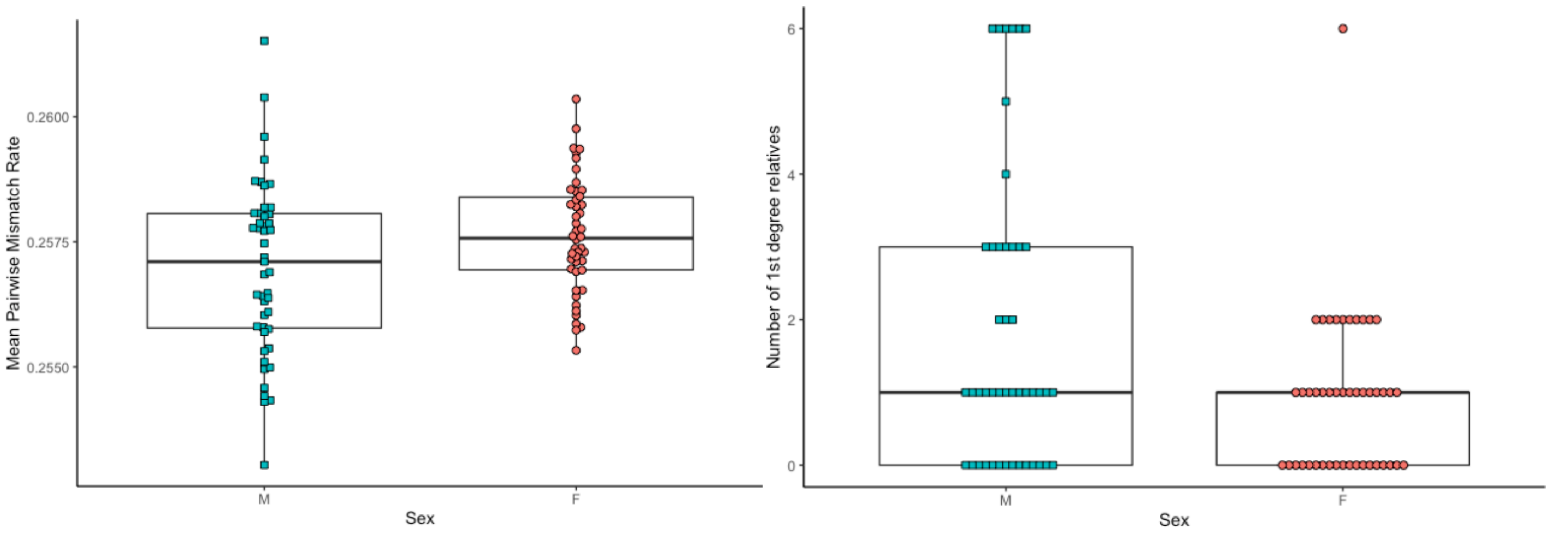


Fig. 4. Intra-site relatedness at Mikulovice. (Left) Mean pairwise mismatch rate between each individual and every other individual buried at Mikulovice. Females tend to have higher values, reflecting a general lower level of relatedness to everyone at the cemetery. (Right) Males tend to have more first-degree relatives buried at Mikulovice than females.

degree. Fifteen males have at least three first degree relatives compared to only one female (Fig. 4).

We observe a higher incidence of males (n=12) than females (n=5) among adults who have at least one parent buried at the site. Although not statistically significantly different from an equal ratio (p=0.143), the observation is consistent with males more likely to remain in their "home community" than females, or at least be buried there. This is in contrast to an even sex ratio observed among individuals younger than 21 (males n=8, females n=8). This is corroborated by the finding of statistically significantly (p=0.004) more individuals with at least one paternal grandparent (n=16) buried at the site compared to those with at least one maternal grandparent (n=3).

Due to the large number of missing inferred individuals in our reconstructed kinship trees, continuous lines of multigenerational paternal or maternal descent where every individual is identified are rare. When found (n=2), they occur over three generations and, in both cases, involve patrilineal descent (biological kinship groups 3 and 9). Multigenerational matrilineal descent is inferred only in two kinship trees (kinship group 3 mtDNA lineage K1a1b2a and kinship group 5 mtDNA lineage H10e), however with two and one unidentified females, respectively. Such a pattern of longer continuous paternal lines of descent is consistent with more patrilocal behaviour (Mittnik et al. 2019).

Our reconstructed kinship trees also offer insights into aspects of social behaviour. The number of children that parents had varies between one and six. Biological kinship group 1 consists of a father and six children, five of whom are male. We have no evidence for either parent having children with other partners, suggesting a long-term (biological) relationship between the two. Curiously, the mother of those six children, who had strong biological ties in Mikulovice through her partners and children, is not identified in the cemetery.

Our kinship trees reveal at least six cases of individuals having offspring with different partners. In four cases it is men having kids with two different women and in two cases it is women having kids with two different men. This is observed twice in biological kinship group 3, for one man and one woman. Interestingly, the woman in this case has one offspring with each of two brothers. Another instance of a woman having children with two different men can be seen in kinship group 5. Kinship group 5 also contains the richest amber grave in EBA Europe (MIB009 – grave 2) and one of only two cases of multigenerational inheritance of mtDNA (lineage H10e).

Stable isotope outliers and biological kinship

Strontium and oxygen isotope analyses of the first molar have revealed approximately 10% of the individuals buried at Mikulovice to be non-locals (Ernée et al. 2020). Among the 14 potential outliers identified by stable isotope analyses, 13 have yielded enough DNA preservation for ancient DNA analysis.

The two most obvious strontium outliers (MIG012 – grave 99 and MIB005 – grave 64a) are individuals who also have no relatives buried at Mikulovice, consistent with them being newcomers to this community. MIG012 (grave 99) is also the only man carrying Y haplogroup G2, a rare lineage previously found in only one other male from Bohemia (Papac

et al. 2021). In general, most individuals (7/8) who have been identified as strontium outliers fall into one of two categories. They are either individuals who have no relatives at the site (n=4), or females who only have one offspring and no other biological relationships (n=3).

Individuals who have been identified as oxygen outliers (n=5) show a different pattern. Each oxygen outlier individual has at least 5 relatives and forms part of one of the four largest biological kinship groups at Mikulovice (biological kinship groups 1, 3, 6, or 9). Most oxygen outliers (4/5) are adolescent or adult males (17+), while one is a young girl (4-5). The finding of individuals who are oxygen isotope outliers, but also biologically well integrated into the community, suggests that at least some individuals who were born in Mikulovice spent a significant portion of their childhood away, eventually to return. Biological kinship group 3 has two oxygen outliers, one from each end of the oxygen isotope distribution, suggesting that some individuals were spending their childhoods in different regions, before being buried at Mikulovice.

Discussion

Our reconstructed kinship groups, in conjunction with archaeological, isotope, and anthropological analyses have allowed for new insights into the lifeways of an EBA community on the Amber road.

In contrast to previous research, a low incidence of stable isotope outliers is observed at Mikulovice, especially unexpected given the large amount of “exotic” grave goods found in burials (Ernée et al. 2020). The newly presented genetic data is largely consistent with this finding, revealing a genetically homogeneous community existing at the intersection of important ancient trade routes in central Europe. However, this homogeneity may not have been the case everywhere in Bohemia during the classical Únětice phase. For example, although data from only twenty-five classical Únětice individuals exist from other sites in Bohemia, four of them (16%) fall outside of the variation sampled at Mikulovice (VLI051, VLI050, PMI009, KO1009, Fig. 2A), suggesting that other sites in Bohemia may have been more influenced by the arrival of non-locals. Interestingly, although only having eight classical Únětice individuals sampled from Vliněves, two of these appear to be genetic outliers, one of them (VLI051) with a clear Baltic genetic profile. This is consistent with previous findings of impressive genetic diversity at Vliněves (Papac et al. 2021), possibly related to its important location at the confluence of the Elbe and Vltava rivers.

In the case of individuals with a non-local stable isotope signal from Mikulovice, they appear to fall within the genetic diversity found in the central European EBA, suggesting that they likely had origins geographically not too far from Bohemia. Interestingly, when combining isotope and genetic data, we find evidence of people who seem well integrated into the Mikulovice community (i.e. having 5+ intra-site relatives, including parents and/or grandparents), but with non-local oxygen isotope signatures. Some of the oxygen isotope outliers have been suggested to have spent their childhood northeast of Bohemia (MIB010 – grave 10, MIB066 – grave 60, MIB083 – grave 86), consistent with the genetic origin of the outlier individual from Vliněves (VLI051). Although connections to the northeast are also suggested for these individuals from Mikulovice, this appears to have been achieved through a different process. In contrast to VLI051, who likely represents a migrant from the

northeast, these three individuals are more likely to have been born in Mikulovice, spent some of their childhood in the northeast, and then returned to Mikulovice. All three, along with VLI051, are male and may represent connections to the northeast related to the origin of Baltic amber in Bohemia.

Although males and females are well represented among isotope outliers at Mikulovice (Ernée et al. 2020), several aspects of the new genetic results suggest a higher incidence of female mobility. These include the larger genetic diversity in females (Fig. 2B, especially in PC2), the overrepresentation of females (8/11) among individuals without close relatives at Mikulovice, the lower average relatedness of females to other individuals within the cemetery (Fig. 4), the lower number of first-degree relatives in females (Fig. 4), the statistically significantly higher incidence of paternal grandparents found, and the lack of multigenerational mtDNA inheritance at Mikulovice. These findings are largely consistent with analyses on contemporaneous groups from the Lech valley (Knipper et al. 2017, Mittnik et al. 2019).

Overall, our results reveal a wealthy EBA cemetery the majority of which was likely used for less than 150 years and made up of a genetically homogeneous community of largely local individuals. Based on the incidence of missing individuals in our kinship trees, the individuals buried at Mikulovice likely formed part of a larger community where some of the missing individuals are likely buried. This community was more patrilocal than matrilocal, expressed a variety of kinship (both biological and social) in their mortuary practices and practiced diverse sexual behaviours, ranging from having six children with the same partner to having offspring with different partners. Patterns of mobility may have included people born locally, who subsequently spent time away, followed by returning to their region of birth.

Materials and methods

Lab work

Teeth and petrous bones from the Mikulovice graves which yielded promising macroscopic skeletal preservation were sampled for ancient DNA. Samples were prepared and processed at the ancient DNA facilities at the Max Planck Institute for the Science of Human History in Jena, Germany, following the protocols described in Papac et al. 2021 (manuscript A). Samples were screened for ancient DNA preservation and DNA libraries with >0.1% human DNA were captured for 1.2 million ("1240K capture") nuclear ancestry informative markers (Mathieson et al. 2015) and mitochondrial DNA. Post-capture libraries were sequenced on an Illumina Hi-Seq or Next-Seq platform using either single end 75 or paired end 50 sequencing setups to a depth of 20-50 million reads per library.

Bioinformatic processing

Sequence data was processed through EAGER (v1.92.38) (Peltzer et al. 2016). Both single end and paired end sequencing runs had their adapters removed via AdapterRemoval (v2.2.0) (Schubert et al. 2016), with paired end sequencing reads also going through a merging step when 11 base pairs of the same sequenced molecule overlapped. Resulting reads were filtered for sequence length (minimum 30 base pairs) and mapped to the human reference genome (hg19) using BAW-aln and BWA-samse (Li and Durbin 2009) using parameters maxdiff (-n) 0.01 and seeding turned off (-l 10000). Duplicate reads were removed using DeDup (v0.12.1) (https://github.com/apeltzer/DeDup) and misincorporation rates in read termini characteristic of ancient DNA damage were assessed using Damageprofiler (v0.3.10) (https://github.com/Integrative-Transcriptomics/DamageProfiler). Last three bases and corresponding base quality scores were masked from BAMs prior to downstream analyses.

Genetic sex determination

The genetic sex of individuals was determined by calculating the normalised mean coverage on the X (mean X coverage / mean autosomal coverage) and Y (mean Y coverage / mean autosomal coverage) chromosomes (Fu et al. 2016). Individuals with normalised mean Y coverage greater than 0.2 were assigned male.

Contamination estimation

Contamination was calculated by using a method which calculates the rate of heterozygousity on the X chromosome (in male samples) (Korneliussen et al. 2014)) and mitochondrial contamination was calculated using schmutzi (for all samples) (Renaud et al. 2015).

Uniparental (mitochondrial and Y-chromosome) haplogroup assignment

Mitochondrial haplogroups were called by creating mitochondrial consensus sequences and using the haplofind web service interface (Vianello et al. 2013). Mitochondrial consensus sequences were produced by creating pileups at each position (map quality and base quality filter 30) followed by calling the most frequent base at each position.
Y haplogroups were called by assessing ancestral/derived alleles from pileups at ISOGG (v15.58 April 2020) positions.

Autosomal genotyping

Samples were genotyped by sampling a random, high quality allele (base quality ≥ 30 and mapping quality ≥ 30) using the sequenceTools (v1.4.0.2) package (https://github.com/stschiff/sequenceTools). Newly generated genotype data was then merged to a compiled set of published published ancient and modern worldwide populations (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data) (v42.2) using mergeit (v2450) from the EIGENSOFT package (https://github.com/DReichLab/EIG).

Ancestry decomposition

Principal components analysis (PCA) was performed using smatpca (v1600) from the Eigensoft packages (https://github.com/DReichLab/EIG). Principal components were calculated on modern day west Eurasian individuals and ancient individuals were projected on the resulting axes.

qpWave and *qpAdm* runs were conducted in admixr v0.7.1 (Petr et al. 2019). Selection of outgroups is indicated in corresponding supplementary table.

Supplementary Information for Manuscript B.

Individuals without close relatives at Mikulovice (n=11)

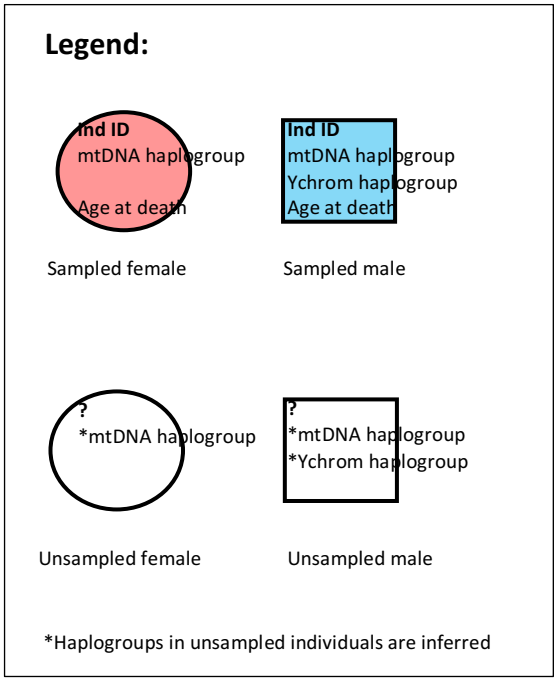
SampleID, Sex, Age at death, Grave **number**, mtDNA haplogroup, Y haplogroup (if male)

MIB005, F, 50+, Grave**64a**, H2a1b
MIB006, M, sen., Grave**64b**, U5a1b1, R1b1a1b
MIB026, F, 35-50, Grave**4**, R0
MIB047, F, adult, Grave**32**, T1a1
MIB055, F, 55+, Grave**44**, H76
MIB007, F, 8-9, Grave**53**, U4c1a
MIB069, F, adult, Grave**65**, U5b2b4
MIG011, F, adult, Grave**98**, R1b
MIG012, M, adult, Grave**99**, J1c1b1a, G2a
MIS006, M, senile, Grave**96**, L1'2'3'4'5'6, R1b-P312
MIS005, F, adult, Grave**94**, K1c1

Fig. S1. Spatial distribution of graves in which individuals without close relatives at Mikulovice were buried.

Biological Kinship Group 1 (n=10)

MIB010, M, 17-19, Grave**10**, K1b1b1, R1b-P312
MIB011, F, 8-11, Grave**11a**, K1b1b1
MIB012, M, 55+, Grave**11b**, J1c, R1b-P312
MIB015, M, 2-4, Grave**19**, K1b1b1, R1b-P312
MIB018, M, adult, Grave**34**, K1b1b1, R1b-P312
MIB030, M, 8-10, Grave**9**, K1b1b1, R1b-P312
MIB035, M, 17-19, Grave**18**, K1b1b1, R1b-P312
MIB033, F, 20-25, Grave **16**, H7b
MIB034, F, 14-16, Grave **17**, H7b
MIB044, F, 50+, Grave **29**, H7b



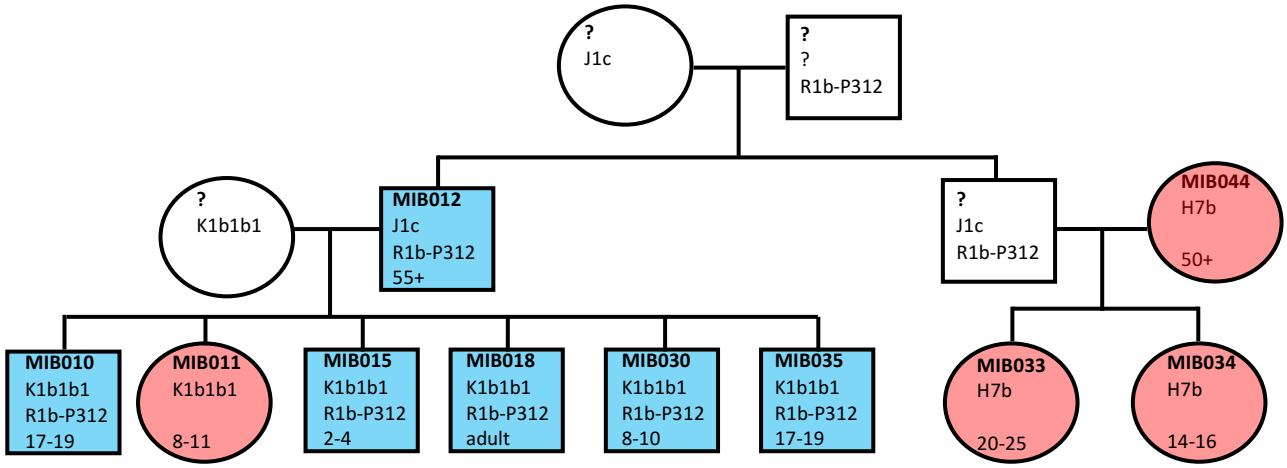


Fig. S2. Pedigree depicting biological relationships of individuals in Biological Kinship Group 1.

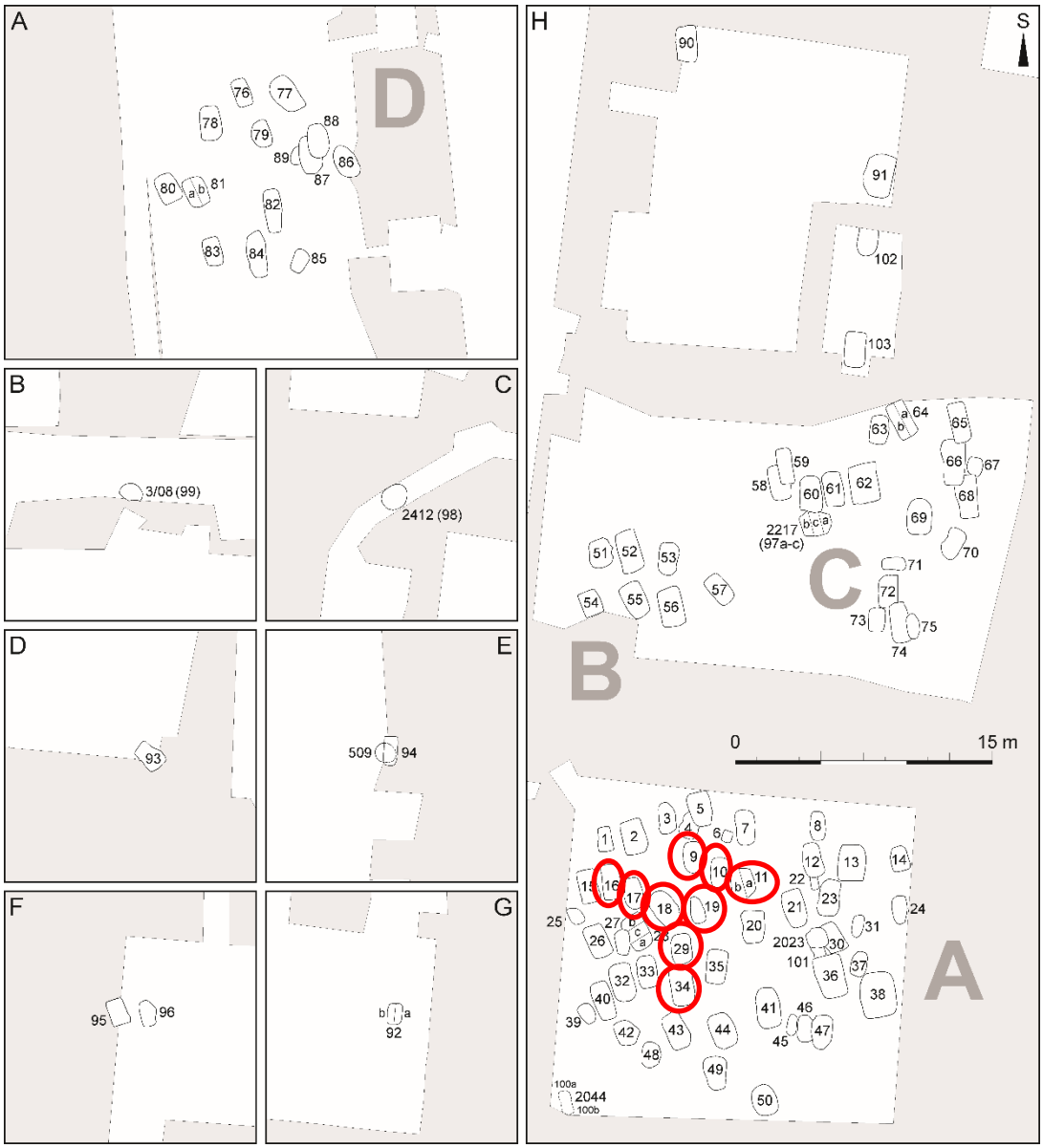


Fig. S3. Spatial distribution of graves in which individuals of Biological Kinship Group 1 were buried.

Biological Kinship Group 2 (n=7)

MIB038, F, 40-55, Grave**23**, V22
MIB045, F, 35-50, Grave**30**, V22
MIB059, M, adult, Grave**49**, V22, R1b-P310
MIB039, F, 4-5, Grave**24**, H27
MIB046, M, 8-10, Grave**31**, T2b, R1b-P312
MIB016, M, 50+, Grave**21**, U5a1g, R1b1a1b
MIB037, F, 5-7, Grave**22**, K1b2b

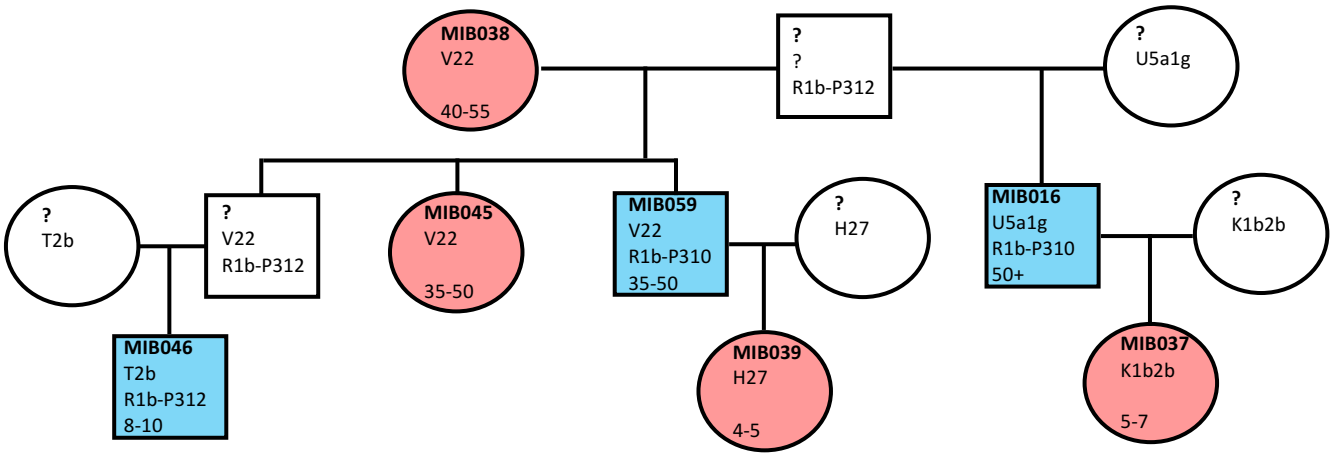


Fig. S4. Pedigree depicting biological relationships of individuals in Biological Kinship Group 2.

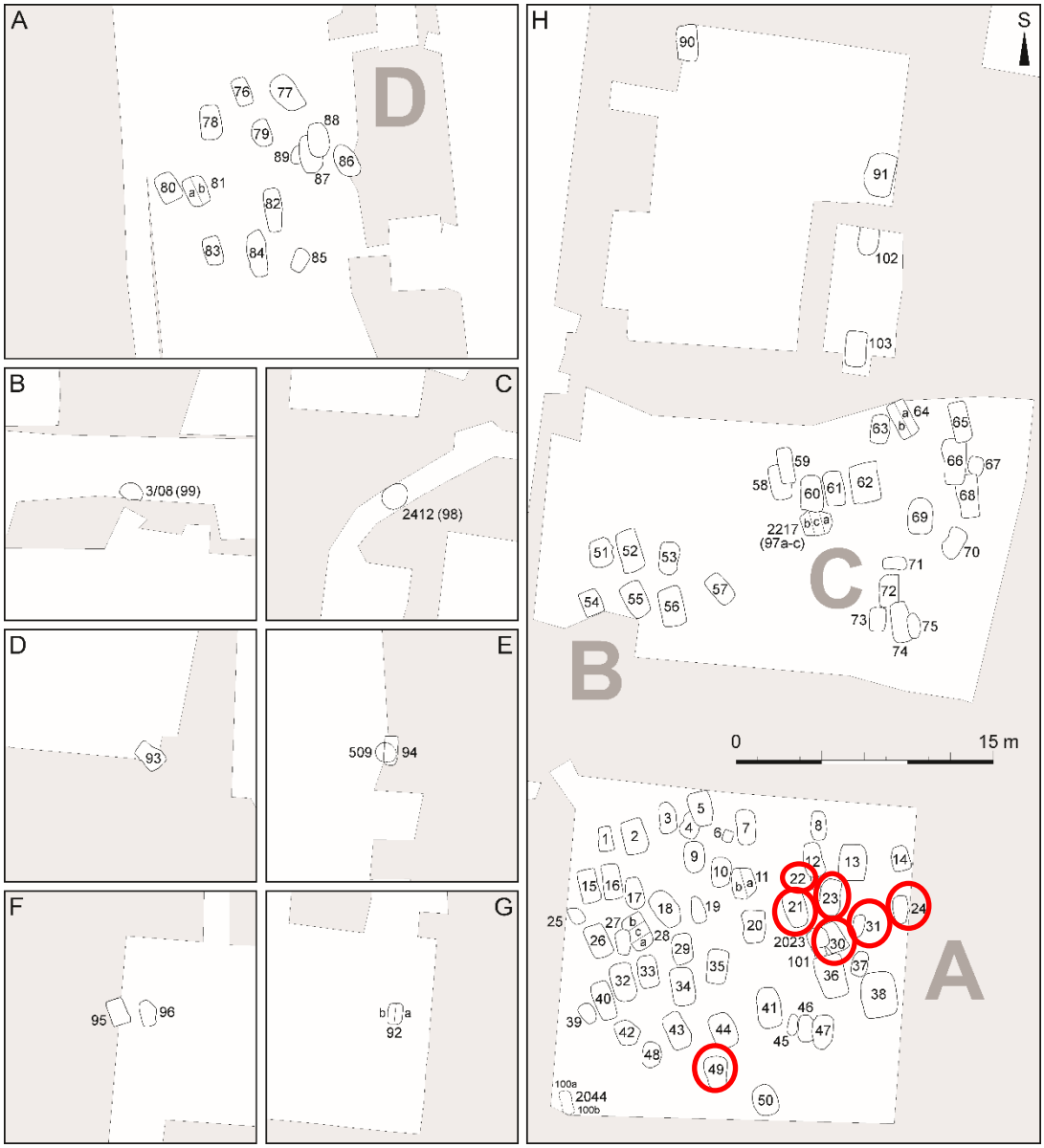


Fig. S5. Spatial distribution of graves in which individuals of Biological Kinship Group 2 were buried.

Biological Kinship Group 3 (n=11)

MIB065, F, 50+, Grave**59**, H11b
MIB067, M, 20-25, Grave**61**, H11b, R1b-P311
MIB066, M, adult, Grave**60**, H11b, R1b-P312
MIB003, F, 15-16, Grave**68**, K1a1b2a
MIB004, M, 20-25, Grave**62**, R1b1, R1b-P312
MIB008, M, 35-50, Grave**69**, H, R1b-P312
MIB068, F, 16-17, Grave**63**, J1c3
MIB071, M, 20-35, Grave**70**, K1a1b2a, R1b-L151
MIB070, F, 4-5, Grave**67**, K1a1b2a
MIB064, M, 50+, Grave**58**, K1a1b2a, R1b-P312
MIB072, M, 16-18, Grave**71** U5b2b2, R1b-P312

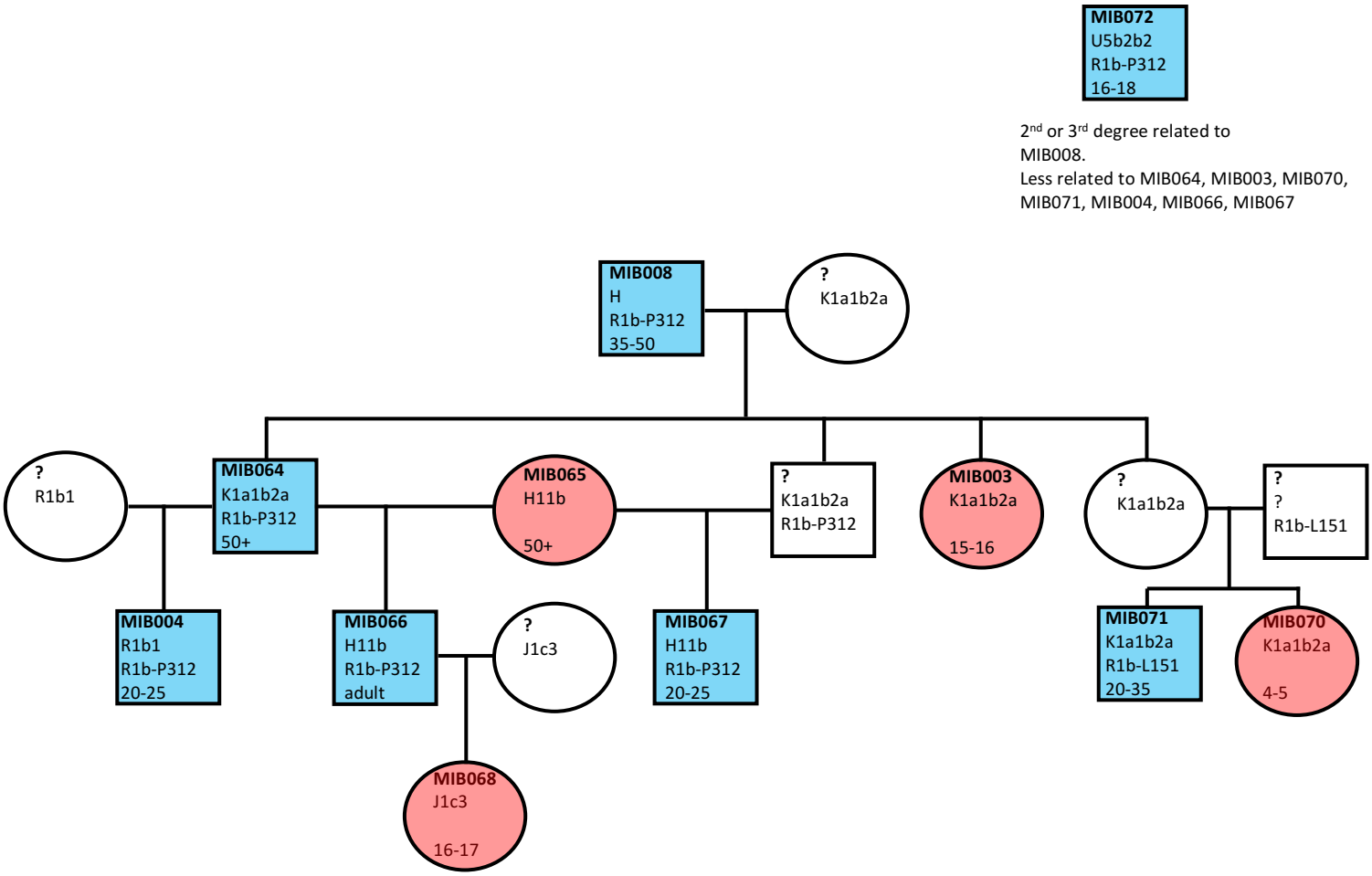


Fig. S6. Pedigree depicting biological relationships of individuals in Biological Kinship Group 3.

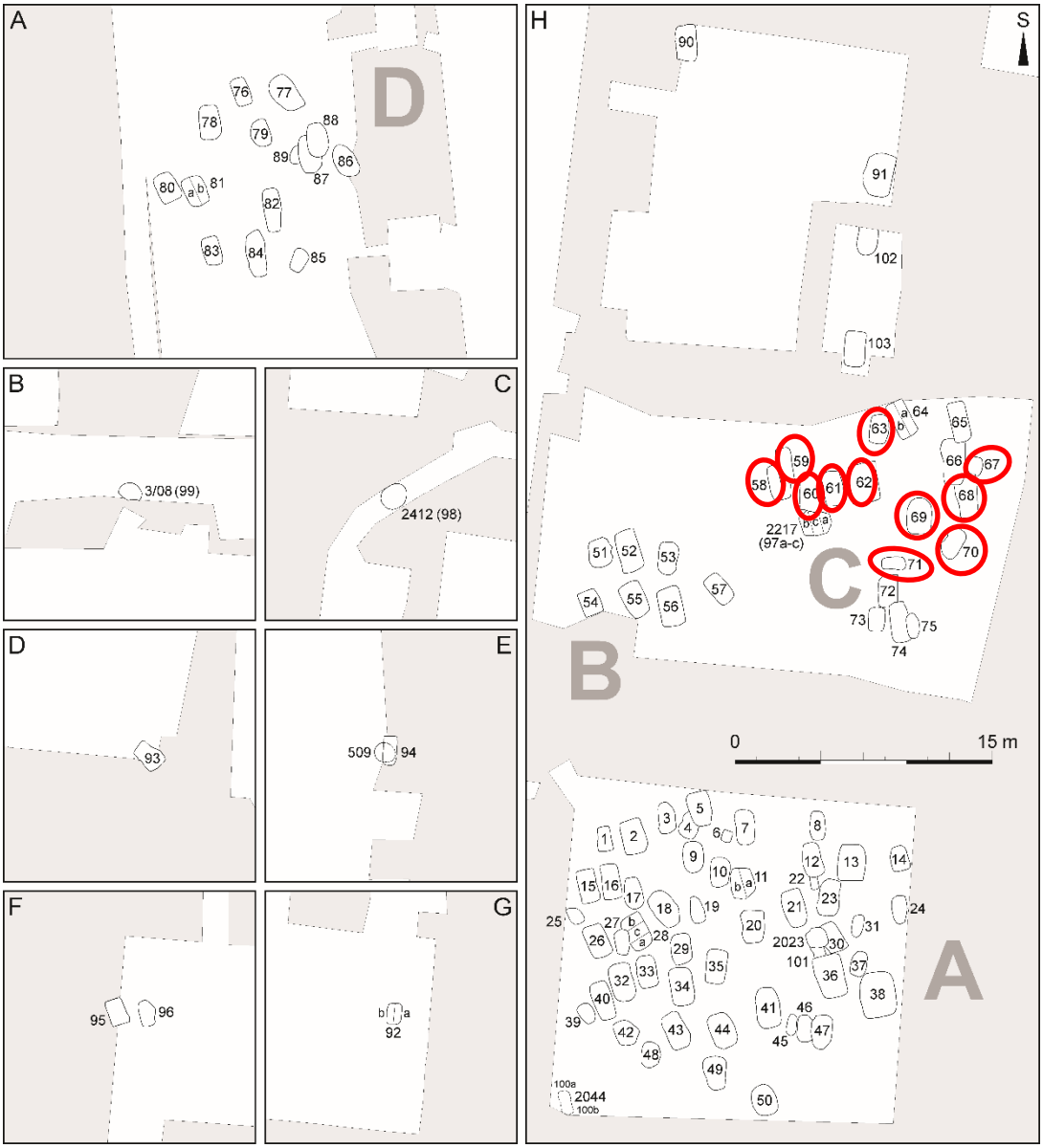


Fig. S7. Spatial distribution of graves in which individuals of Biological Kinship Group 3 were buried.

Biological Kinship Group 4 (n=2)

MIB002, F, 20-25, Grave**55**, U4b1b1
MIB062, M, 50+, Grave**56**, K1c1, R1b-P312

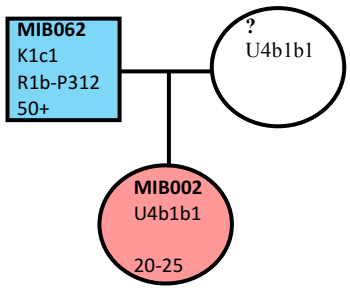


Fig. S8. Pedigree depicting biological relationships of individuals in Biological Kinship Group 4.

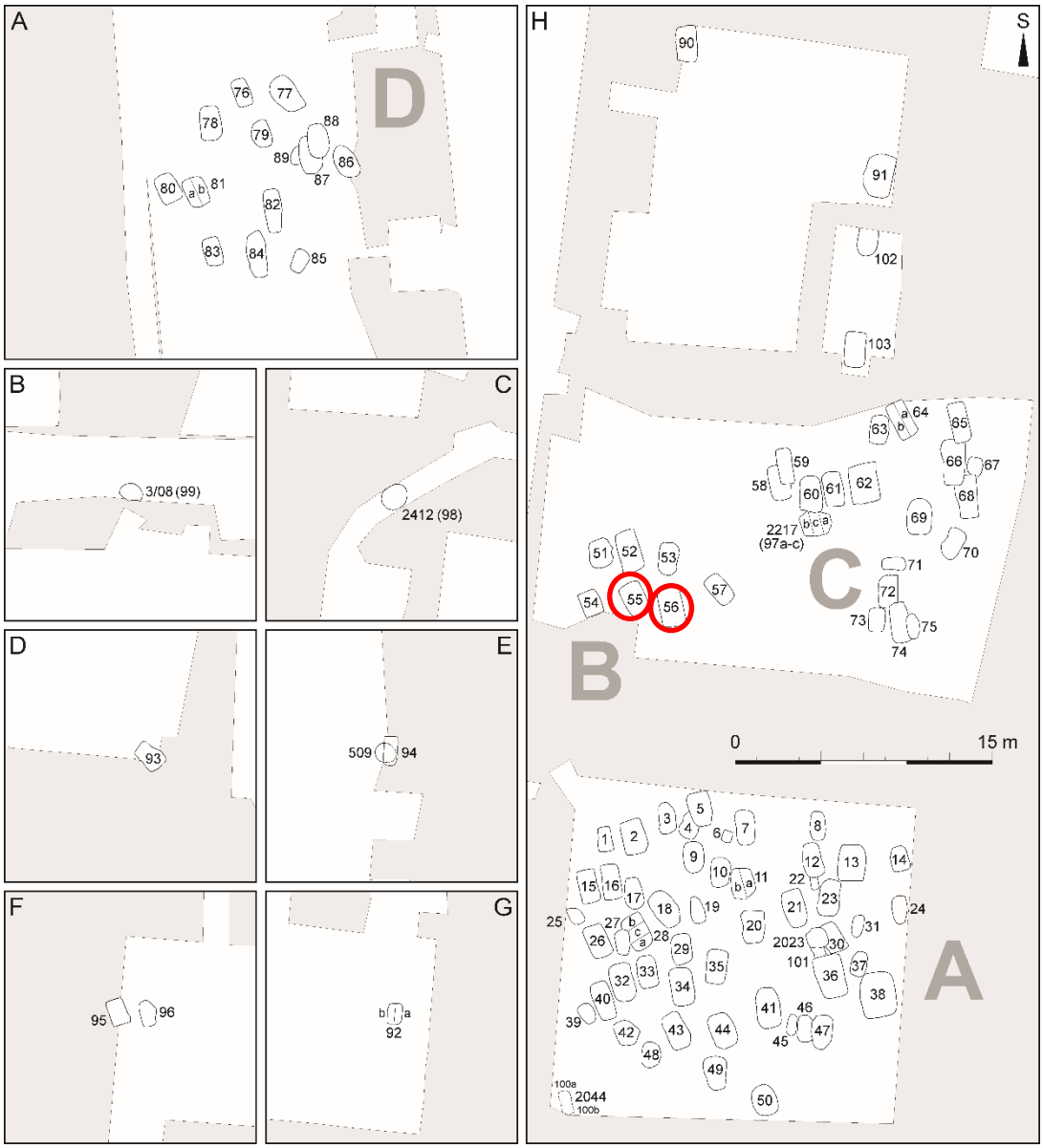


Fig. S9. Spatial distribution of graves in which individuals of Biological Kinship Group 4 were buried.

Biological Kinship Group 5 (n=6)

MIB009, F, 35-50, Grave**2**, H10e
MIB014, F, senile, Grave**15**, H10e
MIB024, F, 5-6, Grave**1**, H1b
MIB040, M, 5-7, Grave**25**, H10e, R1b-P312
MIB019, F, 35-50, Grave**36**, H1b
MIB028, F, 35-50, Grave**7**, U5b2a2c

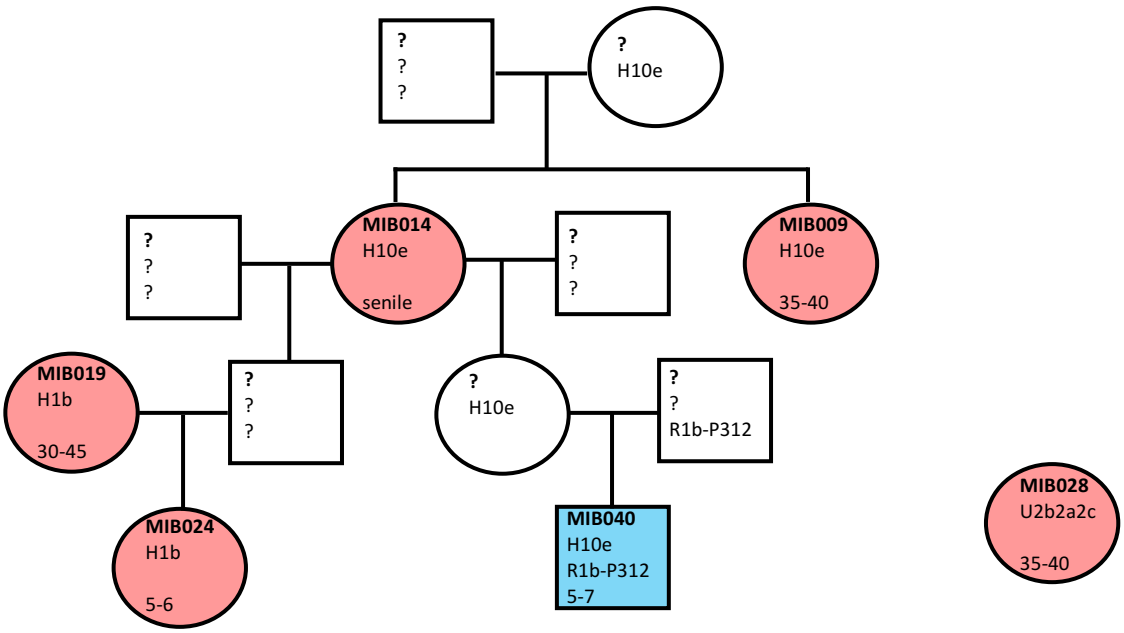


Fig. S10. Pedigree depicting biological relationships of individuals in Biological Kinship Group 5.

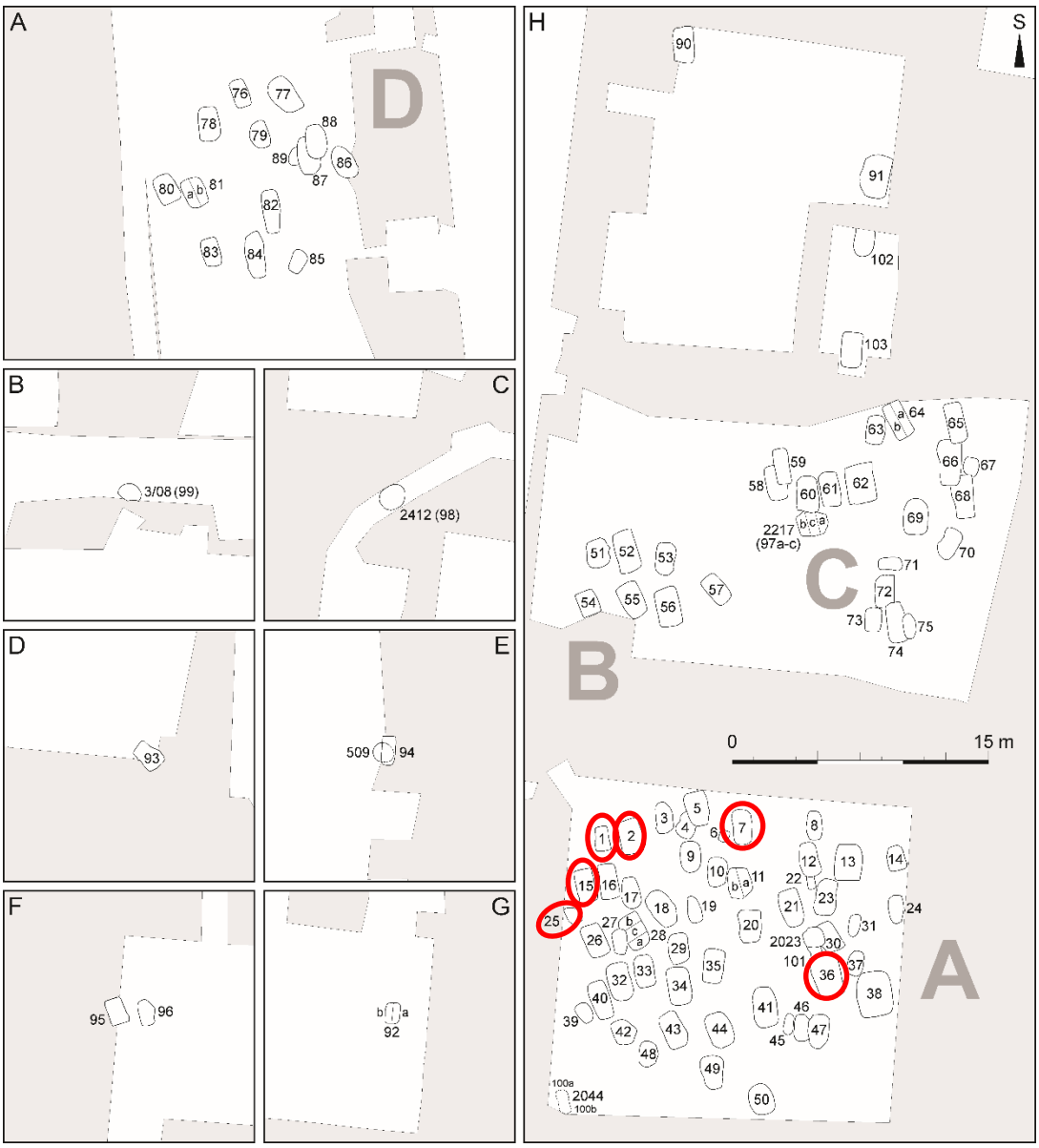


Fig. S11. Spatial distribution of graves in which individuals of Biological Kinship Group 5 were buried.

Biological Kinship Group 6 (n=13)

MIB086, M, 35-50, Grave**90**, K1f, R1b-P312
MIB073, M, 25+, Grave**73**, U5b1b, R1b-P312
MIB074, F, 55+, Grave**74**, U5b1c1a
MIS001, M, 2-3, Grave**92a**, U5b1c1a, R1b-P312
MIS002, M, 6-8, Grave**92b**, T2c1d1, R1b-P312
MIB076, M, adult, Grave**77**, N1, R1b1a1b
MIB077, F, ±20, Grave**78**, R0
MIB083, M, 35-50, Grave**86**, H4a1a1a, R1b-P312
MIB078, F, 50+, Grave 79, H2a2a1
MIB027, M, 25-40, Grave**5**, R1b1, R1b-P312
MIB075, M, 4-5, Grave**75**, U8b1b1, R1b-P312
MIB082, F, 6-8, Grave**83**, I4a
MIB084, M, 12-14, Grave**87**, H2, R1b-P312

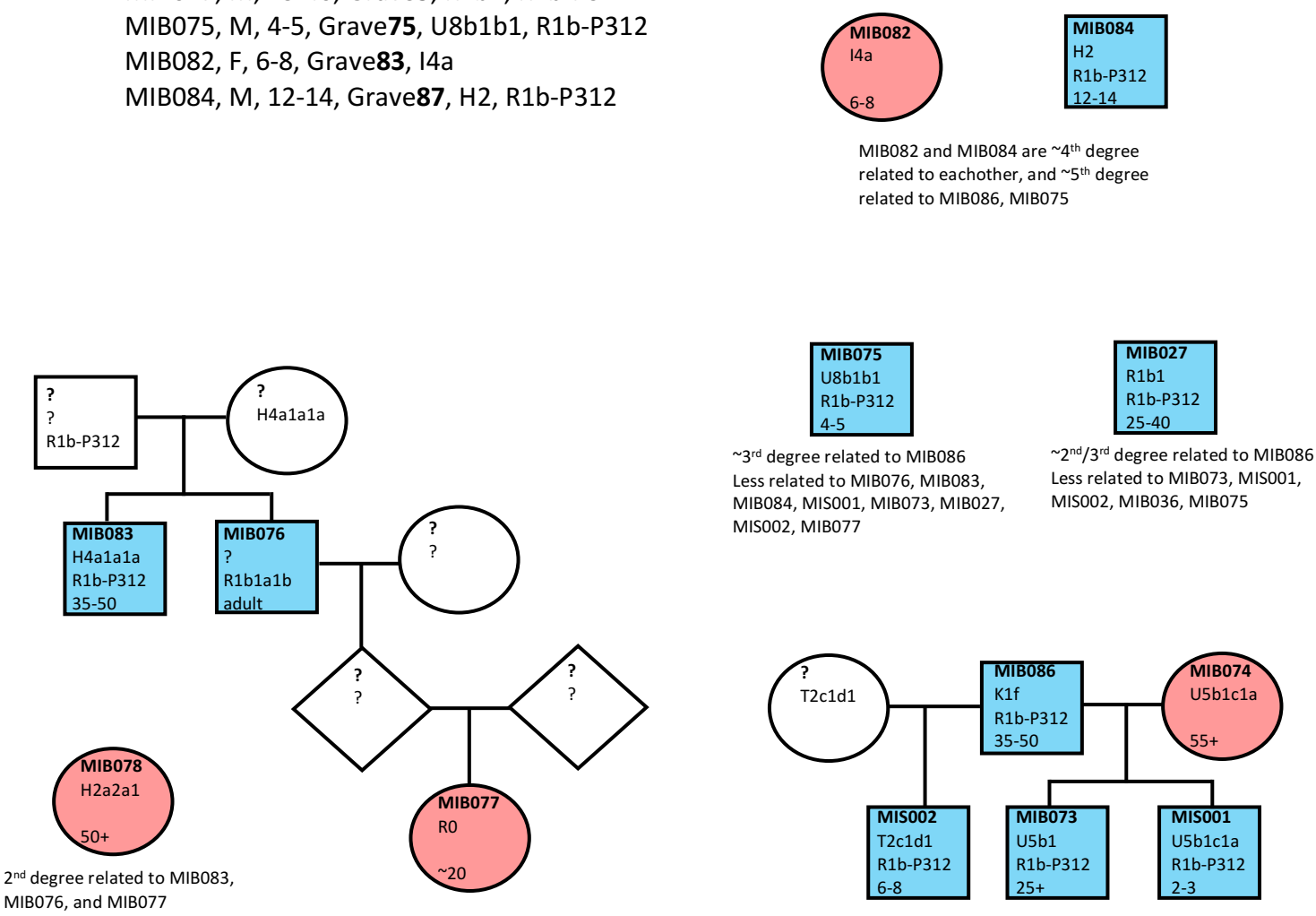


Fig. S12. Pedigree depicting biological relationships of individuals in Biological Kinship Group 6.

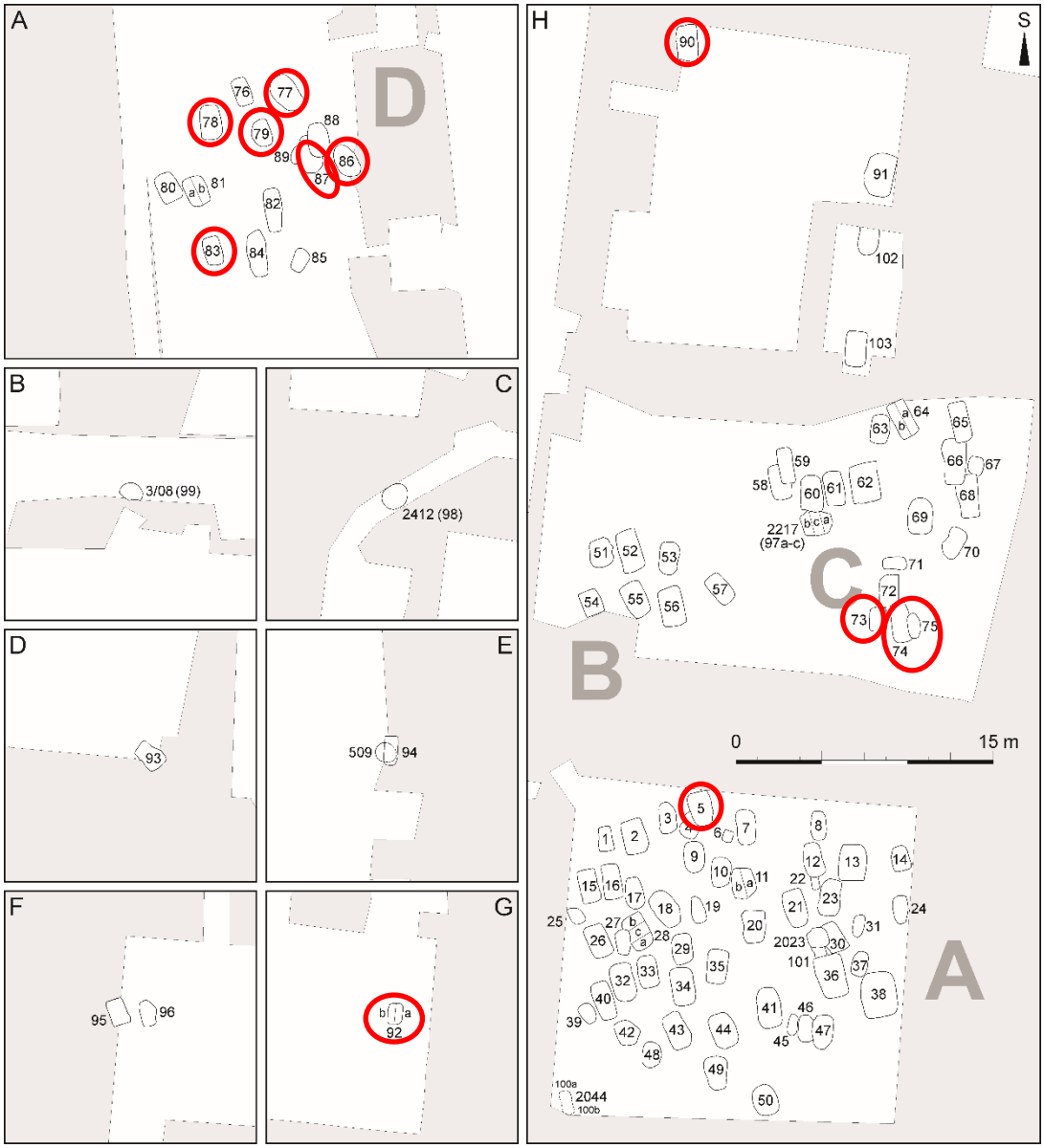


Fig. S13. Spatial distribution of graves in which individuals of Biological Kinship Group 6 were buried.

Biological Kinship Group 7 (n=2)

MIB031, F, 18-20, Grave**13**, HV16
MIB032, F, 55+, Grave**14**, HV16

MIB031 and MIB032 are 2nd degree related. Could be half-sisters, aunt/niece, or grandmother/granddaughter

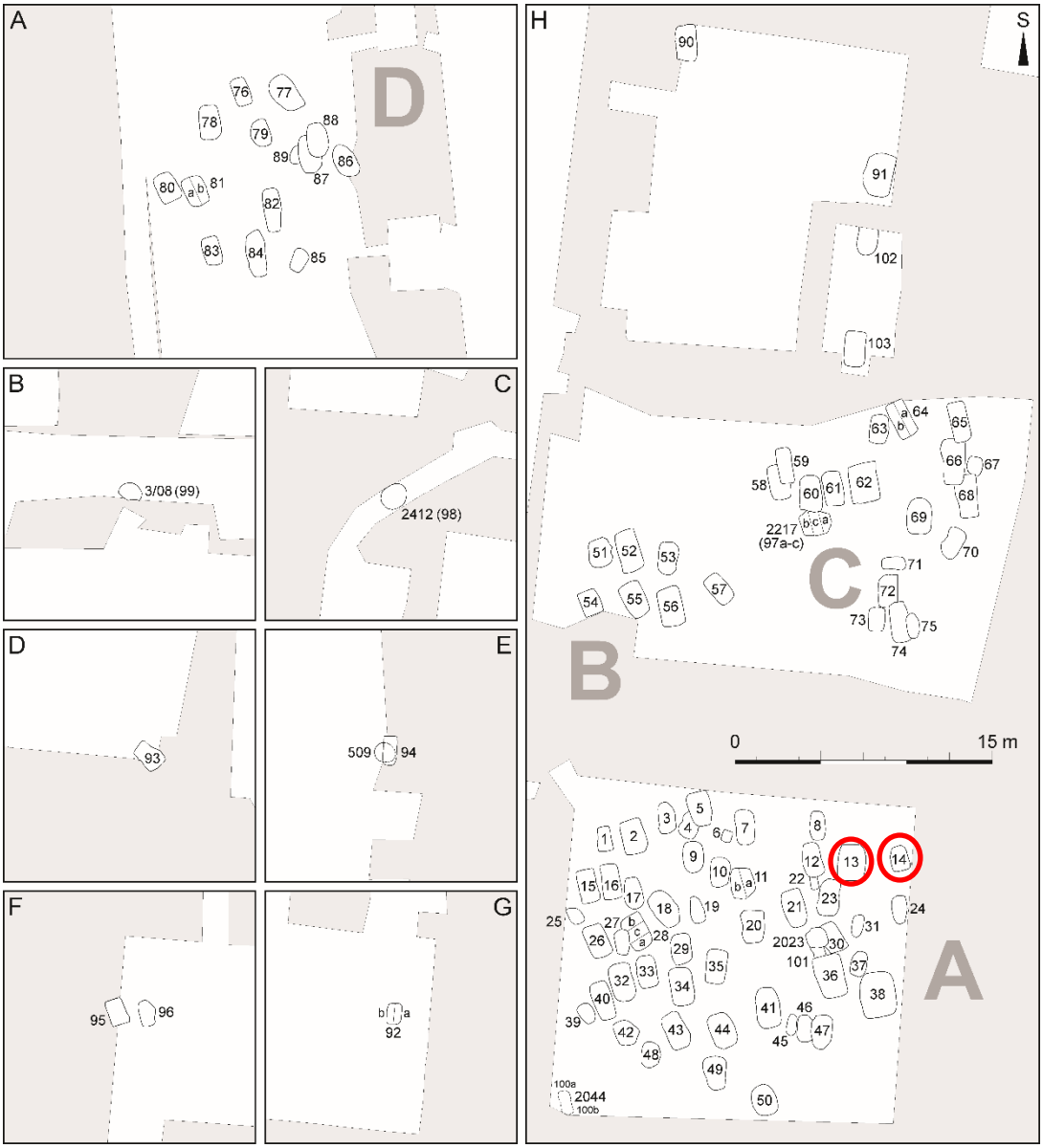


Fig. S14. Spatial distribution of graves in which individuals of Biological Kinship Group 7 were buried.

Biological Kinship Group 8 (n=3)

MIB013, F, 55+, Grave**12**, J1c3
MIB029, F, 20-30, Grave**8**, J1c3
MIS004, M, 20-35, Grave**93**, H17, I2

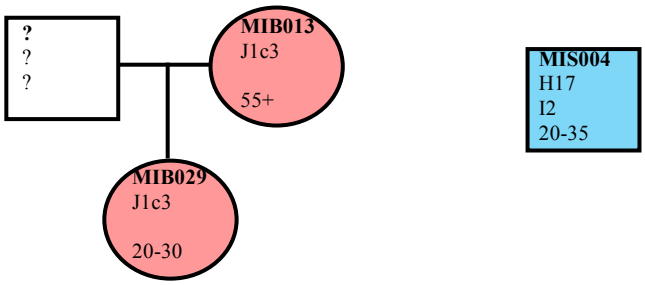


Fig. S15. Pedigree depicting biological relationships of individuals in Biological Kinship Group 8.

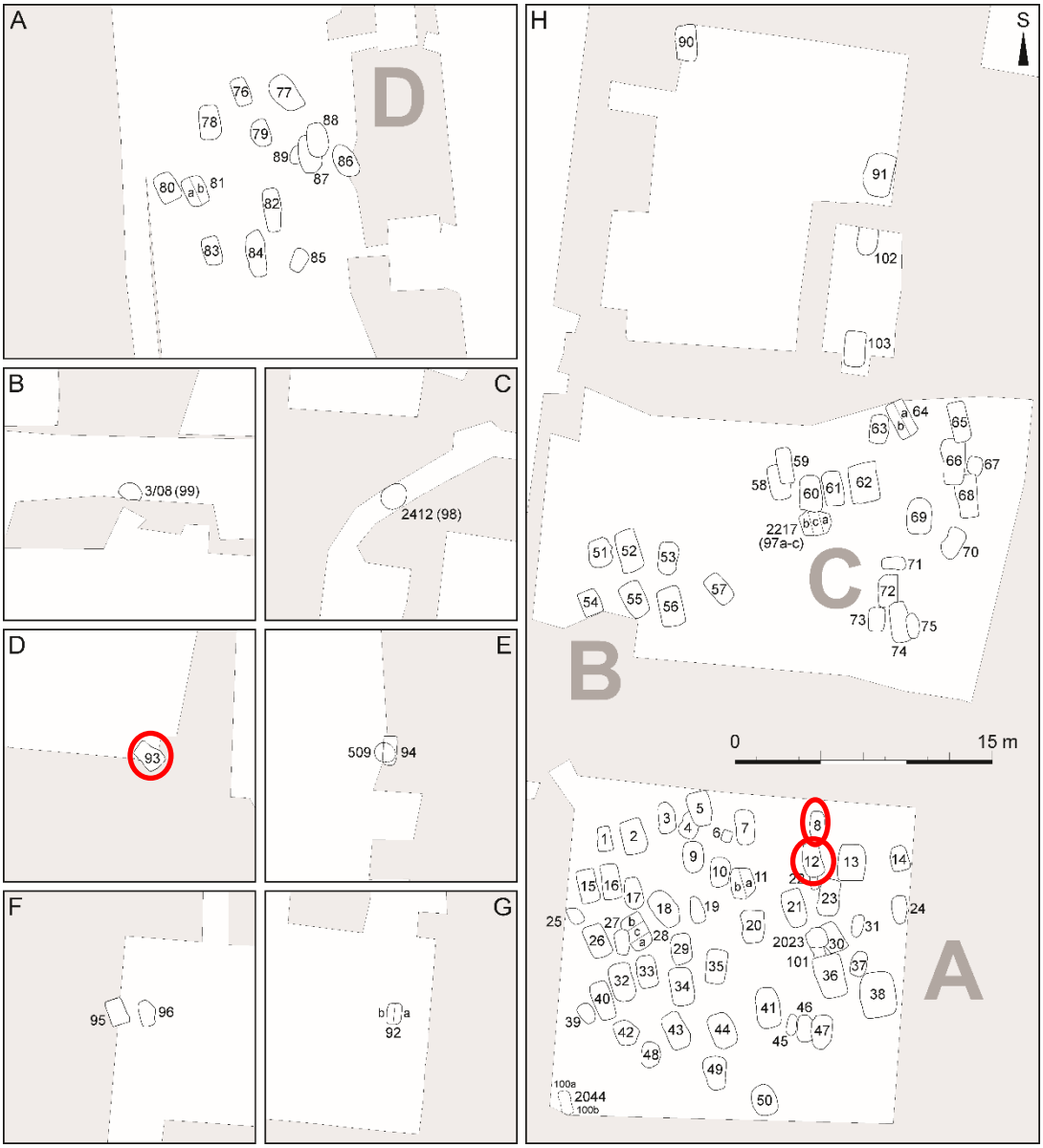


Fig. S16. Spatial distribution of graves in which individuals of Biological Kinship Group 8 were buried.

Biological Kinship Group 9 (n=11)

MIB022, M, adult, Grave**43**, I4a, R1b-P312
MIB043, M, 30-45, Grave**28c**, I4a, R1b-P312
MIB048, F, 55+, Grave**33**, I4a
MIB087, M, 55+, Grave**91**, J1c1b1a, R1b-P312
MIB041, M, 9-11, Grave**28a**, T1a1, R1b-P312
MIB042, F, 6-7, Grave**28b**, T1a1
MIB052, F, 35-50, Grave**40**, U2e1
MIB017, M, senile, Grave**26**, U2e1, R1b-P312
MIB054, M, 6-7, Grave**42**, W1, R1b-P312
MIB020, F, 4-5, Grave**39**, U2e1b
MIB058, F, 5-6, Grave**48**, H6a1a

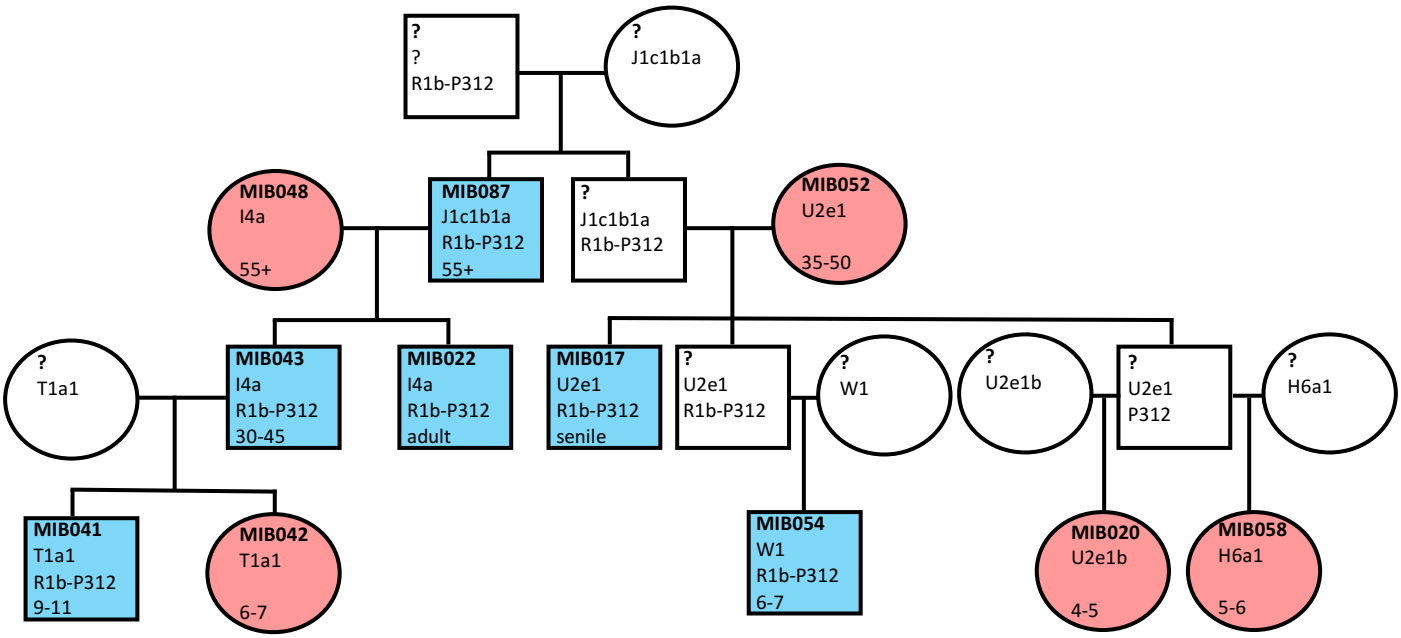


Fig. S17. Pedigree depicting biological relationships of individuals in Biological Kinship Group 9.

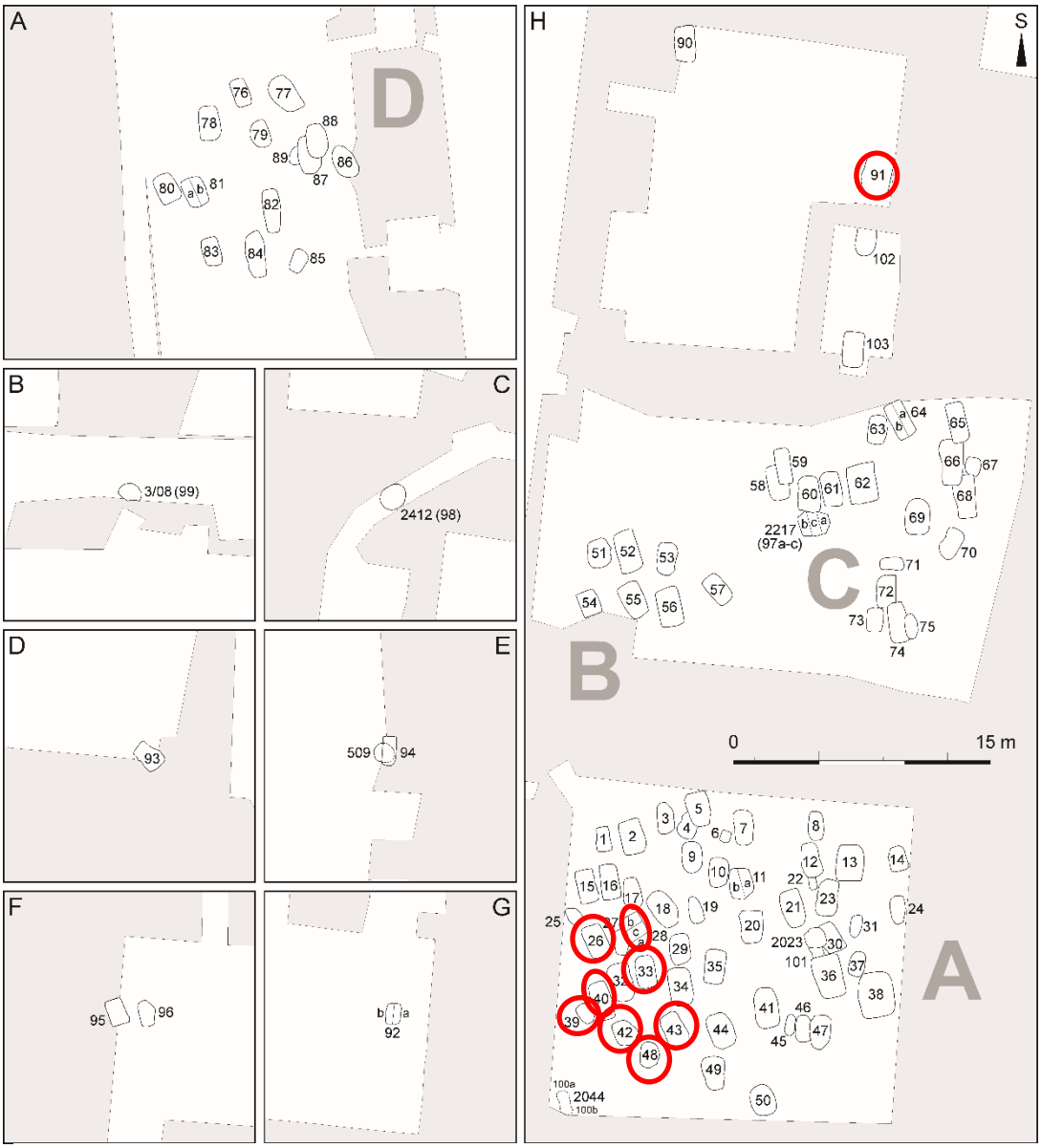


Fig. S18. Spatial distribution of graves in which individuals of Biological Kinship Group 9 were buried.

Biological Kinship Group 10 (n=4)

MIB001, M, 4-5, Grave**51**, T2b, R1b-P312
MIB060, M, 20-35, Grave**52**, H1, R1b-L151
MIB061, M, 30-45, Grave**54**, H1, R1b-P312
MIB063, M, 45-60, Grave**57**, W1c, R1b-P312

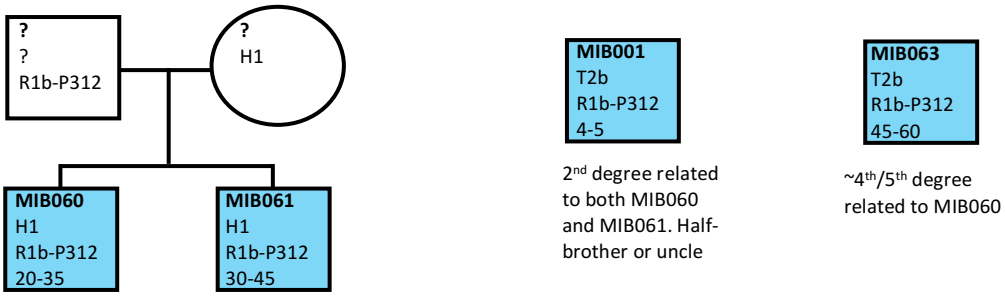


Fig. S19. Pedigree depicting biological relationships of individuals in Biological Kinship Group 10.

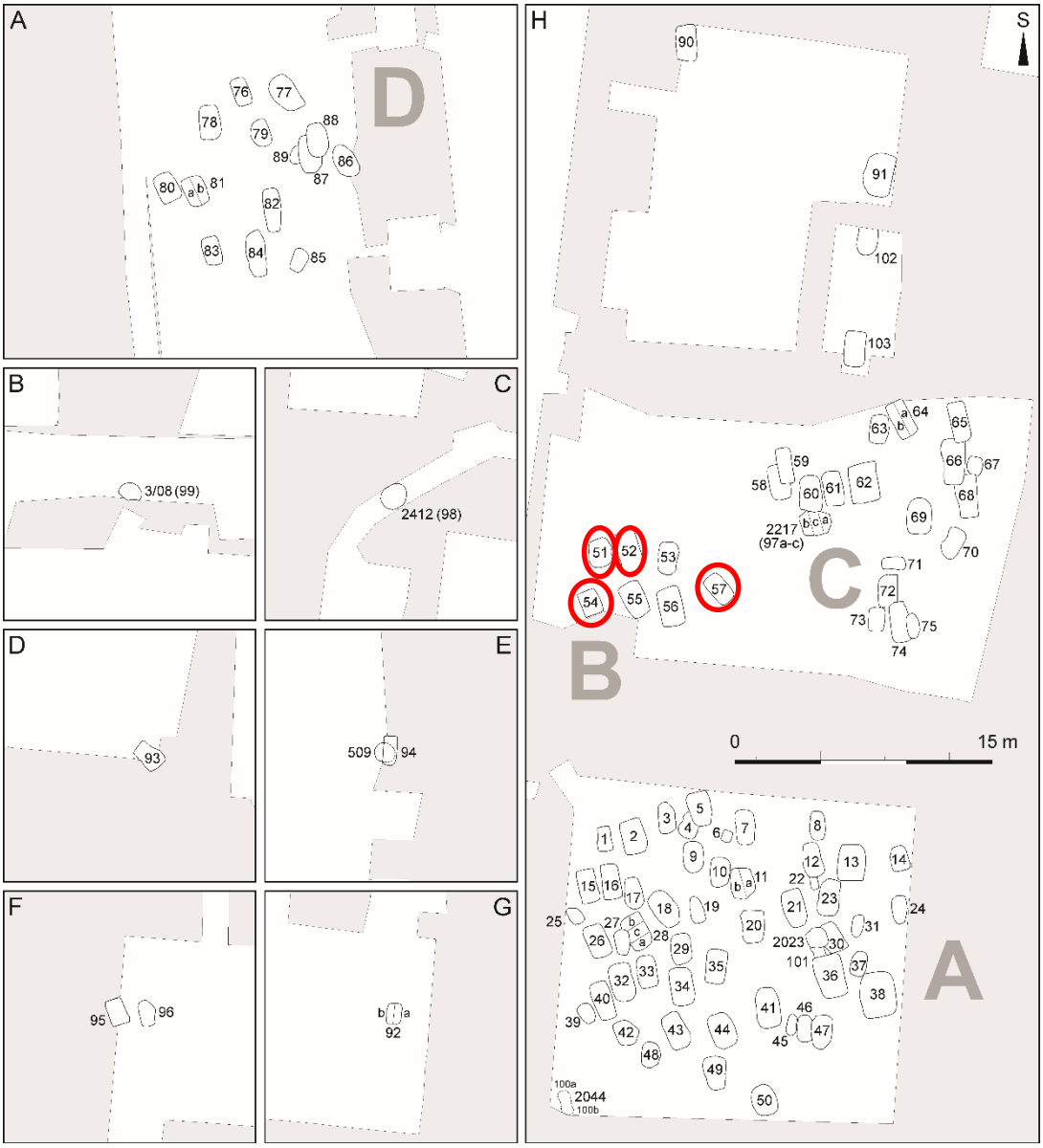


Fig. S20. Spatial distribution of graves in which individuals of Biological Kinship Group 10 were buried.

Biological Kinship Group 11 (n=7)

MIB036, F, 50+, Grave**20**, H6c
MIB049, F, adult, Grave**35**, H
MIB053, M, 40-55, Grave**41**, H6c, R1a
MIB051, M, 25-35, Grave**38**, H1, R1a-Z647
MIB056, M, 3-4, Grave**45**, I, R1a
MIB057, F, adult, Grave**47**, I4a
MIB021, F, 20-35, Grave**50**, T1a1

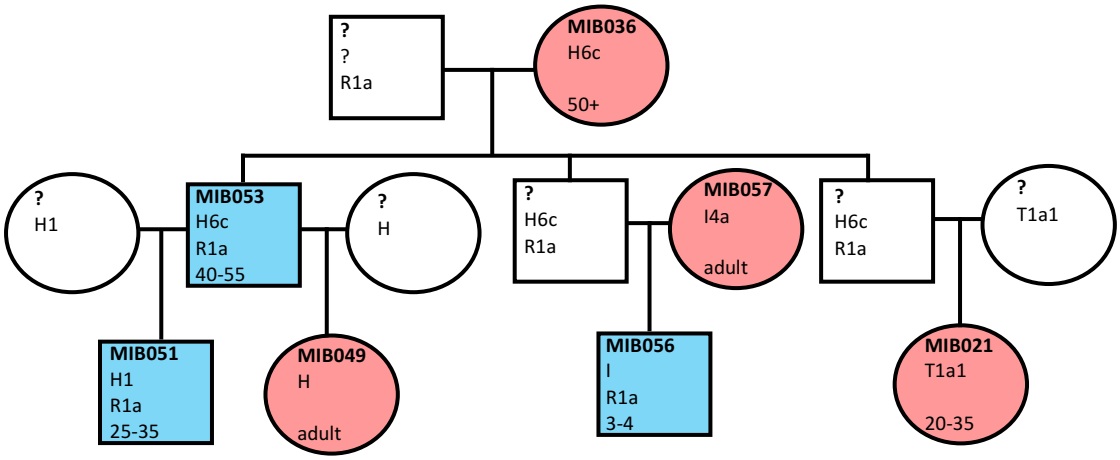


Fig. S21. Pedigree depicting biological relationships of individuals in Biological Kinship Group 11.

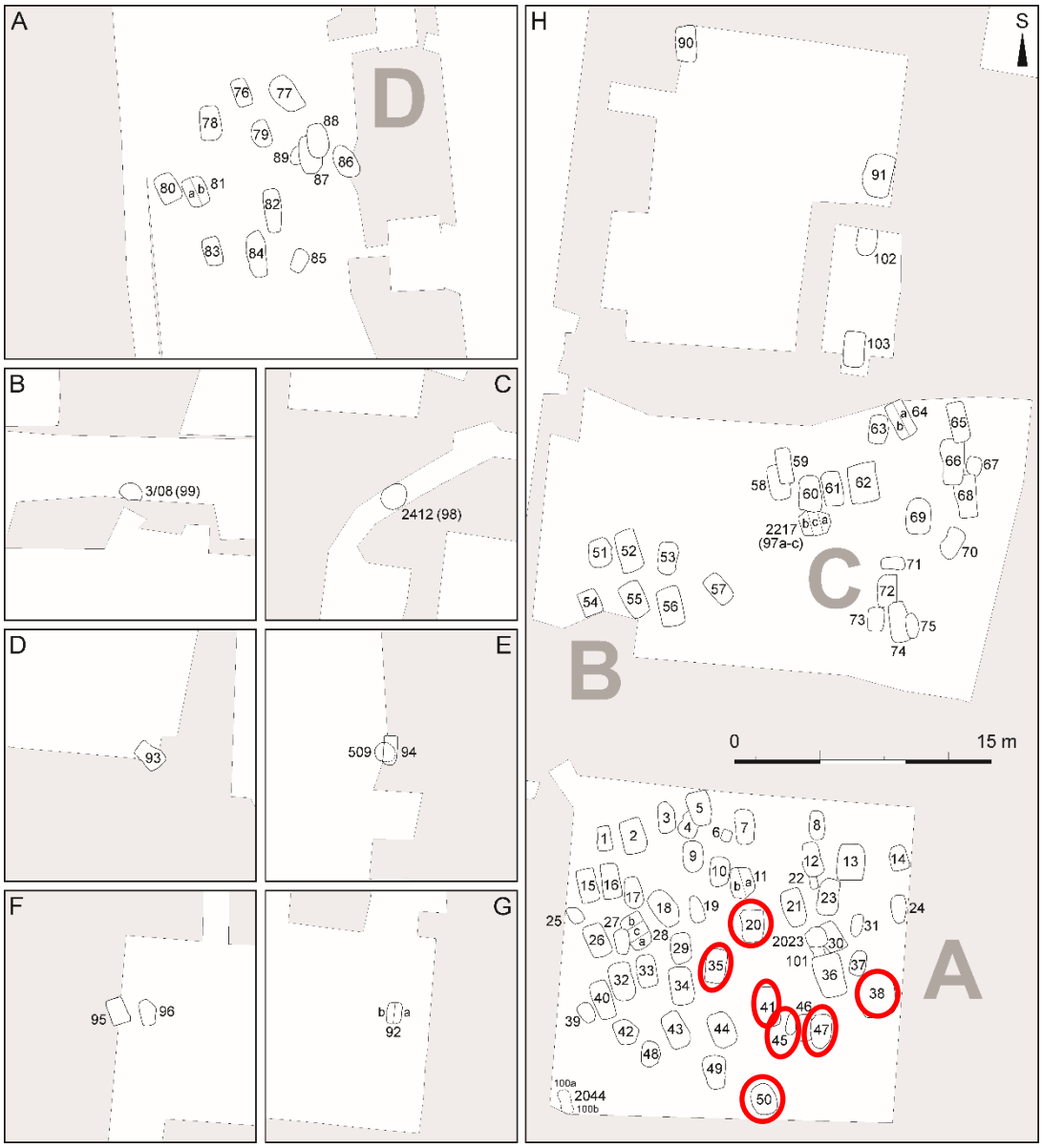


Fig. S22. Spatial distribution of graves in which individuals of Biological Kinship Group 11 were buried.

Biological Kinship Group 12 (n=5)
MIG008, M, ~18, Grave**97a**, T2, R1b1a1b
MIG009, M, adult, Grave**97b**, J1c1b1a1, R1b1a1b
MIG010, F, 55+, Grave**97c**, T2e
MIB079, F, 30-45, Grave**80**, H24
MIS007, F, 12-14, Grave **72**, T2

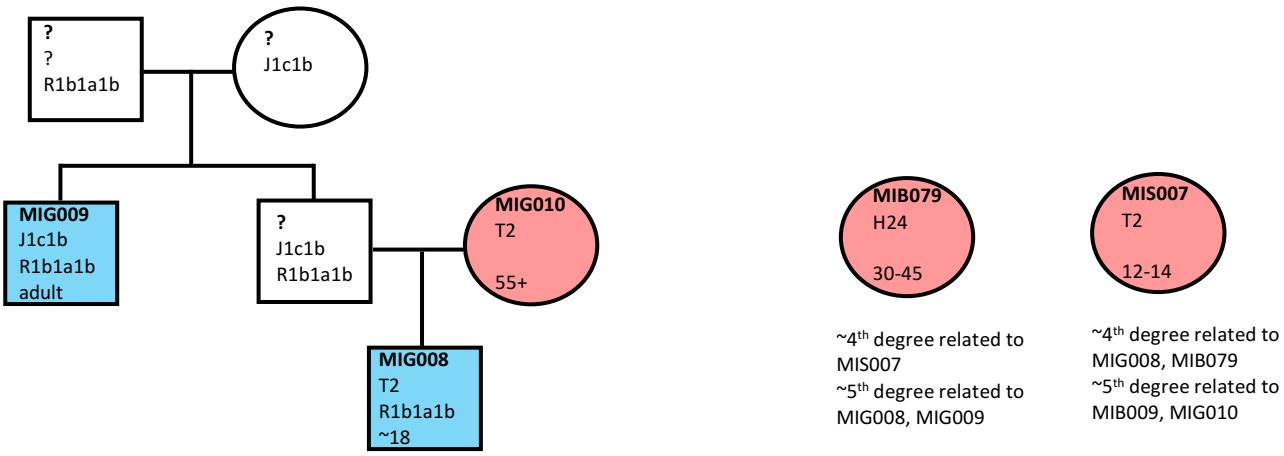


Fig. S23. Pedigree depicting biological relationships of individuals in Biological Kinship Group 12.

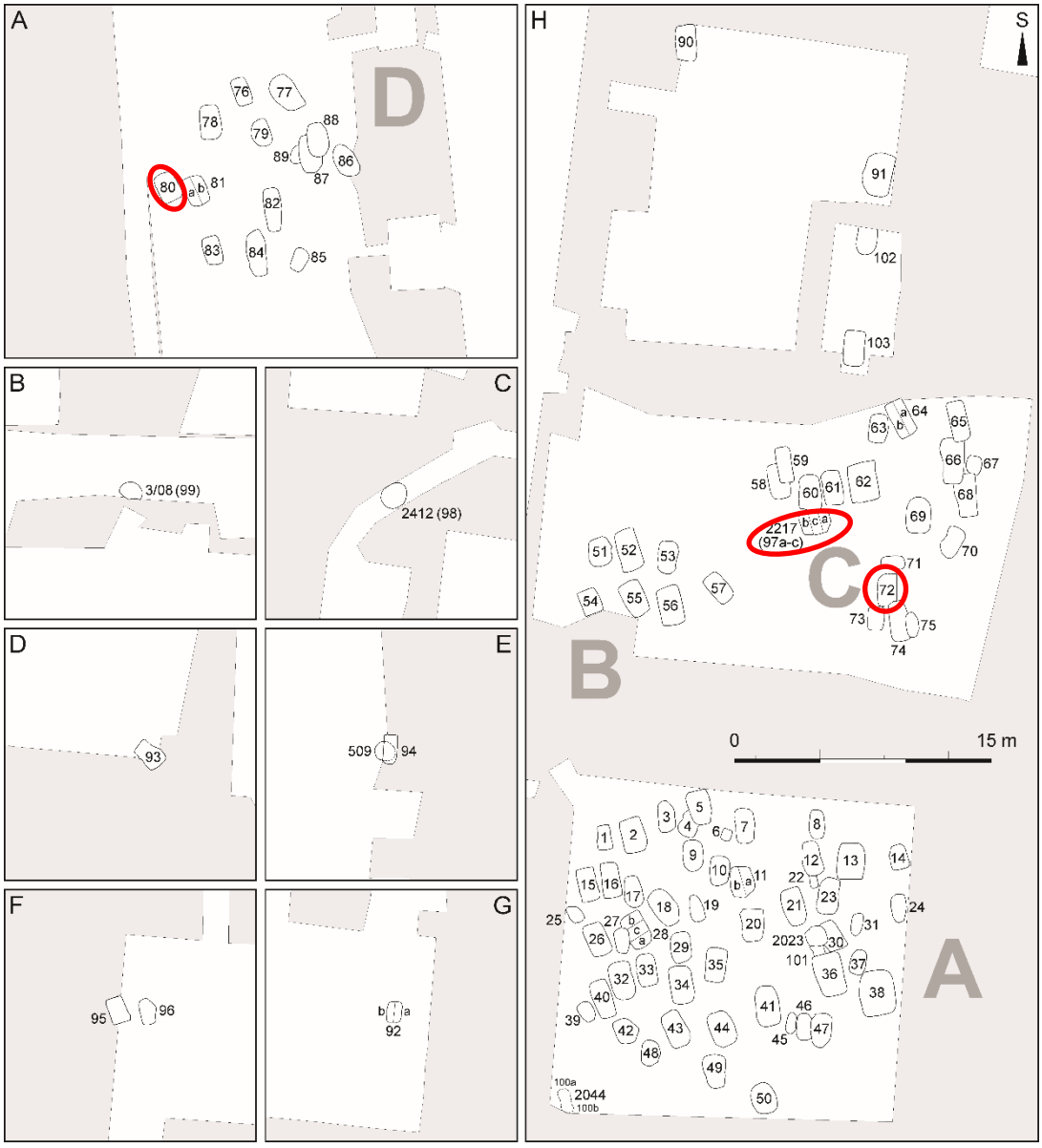


Fig. S24. Spatial distribution of graves in which individuals of Biological Kinship Group 12 were buried.

Table S1-S7. (separate Excel spreadsheet). Supplementary tables are too large and have been burnt onto CD which accompanies this thesis.

Table S1: Contextual information for all sampled individuals from Mikulovice EBA cemetery.

Table S2: Summary 92 individuals analysed from Mikulovice EBA cemetery.

Table S3: Kinship summary table for EBA Mikulovice individuals.

Table S4: List of 1,141 modern West Eurasian individuals on which Principal Components Analysis was conducted (ancient individuals were projected).

Table S5: qpAdm modelling of each EBA Mikulovice individual as a 3-way mixture of Loschbour, Anatolia_Neolithic and Yamnaya_Samara.

Table S6: qpWave cladality test for each Biological Kinship Group.

Table S7: qpWave cladality test for each EBA Mikulovice individual.

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6. Manuscript C

Title: Ten millennia of hepatitis B virus evolution

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Abstract
Hepatitis B virus (HBV) has been infecting humans for millennia and remains a global health problem, but its past diversity and dispersal routes are largely unknown. We generated HBV genomic data from 137 Eurasians and Native Americans dated between ~10,500 and ~400 years ago. We date the most recent common ancestor of all HBV lineages to between ~20,000 and 12,000 years ago, with the virus present in European and South Americans during the early Holocene. Following the European Neolithic transition, Mesolithic HBV strains were replaced by a lineage likely disseminated by early farmers that prevailed throughout western Eurasia for ~4,000 years, declining around the end of the 2nd millennium BCE. The only remnant of this prehistoric HBV diversity is the rare genotype G, which appears to have re-emerged during the HIV pandemic.

One-Sentence Summary:
Uncovering 10,000 years of hepatitis B virus evolution by analyzing genomes from ancient Eurasians and Native Americans

Main Text:

The World Health Organization (WHO) estimates that in 2015, 257 million people were living with chronic hepatitis B virus (HBV) infection, which causes close to one million deaths each year (*1*). HBV is transmitted through contact with bodily fluids, mainly in sexual and perinatal contexts (*2*), and has no known environmental or animal reservoir. Its spread is therefore tightly linked to the dispersal of humans, whose past population dynamics and migrations have likely shaped the genetic diversity of this partially double-stranded DNA virus, which is currently classified into nine genotypes associated with characteristic ethno-geographic ranges [(*3*, *4*); Fig. 1]. However, the temporal and geographic context of HBV origins in humans, as well as its major routes of dissemination in the past, remain widely debated (*5–10*). Recent studies have retrieved HBV DNA from archaeological human remains (*11–16*), providing new avenues to address questions about HBV evolution and phylogeographic history. In particular, these studies revealed the presence of HBV in Europe as early as the Neolithic and ancient HBV lineages that are now seemingly extinct. Ancient DNA data permits molecular clock calibration, and the time to the most recent common ancestor (tMRCA) of all known HBV lineages has been dated to between ~21 and ~9 thousand years ago (ka) (*14*). However, the extent of the past diversity of this virus remains generally unknown as only 19 ancient HBV genomes with a limited temporal and geographic distribution have been reconstructed to date.

The MRCA of all known HBV lineages

Here, we report genomic evidence of HBV in the skeletal remains of 137 individuals from Eurasia and the Americas dated to between ~10,500 and ~400 years ago (Figs. 1, S1; Data S1). Despite advances in molecular virology and numerous sequences from present-day HBV genomes, assessing the phylogenetic relationships among HBV genotypes has proven challenging (*7*, *17–20*), and doubts have been cast about its evolutionary rate and molecular clock-like behavior (*9*, *16*, *21*). Nevertheless, most HBV phylogenetic reconstructions have recovered a topology in which HBV genotypes typically found in Native Americans (F and H) represent a sister clade to the rest of worldwide HBV diversity (*18*) (which we refer to as the Eurasian branch). This topology was supported by a study incorporating 12 ancient HBV genomes (*14*), and is retrieved here (Figs. 2, S2, S3). In particular, the monophyly of the American HBV branch, comprising all ancient genomes from the Americas dating back to as early as ~9 ka from the Cuncaicha rock shelter in the Andean highlands (CUN002), was highly supported. On the other hand, deep nodes within the Eurasian branch were not well resolved, pointing to plausible alternative topologies in which some of the earliest Eurasian lineages would have diverged before the American branch [see (*22*); Figs. S4, S5]. Our results confirm that HBV genomic data do exhibit a clear temporal structure when incorporating samples spanning several thousand years (fig. S3). Using the best-fitting uncorrelated relaxed clock model, we estimate the tMRCA of HBV, corresponding to the divergence of American and Eurasian HBV branches, between ~16 and ~12 ka [95% Highest Posterior Density (HPD); table S1], within the range of previous findings (*14*). This suggests that contacts between ancestral Eurasians and First Americans occurred until at least shortly before the Bølling-Allerød interstadial (~15-13 ka), a period of warming corresponding to widespread human expansion in North America (*23*, *24*). However, studies of ancient human genomes indicate that the ancestors of the First Americans likely began diverging from their closest Eurasian relatives between ~25 and 18 ka, possibly reflecting an extended isolation in a Beringian refugium during the Last Glacial Maximum, before dispersing into and across

the Americas (*25*–*27*). The use of a time-dependent rate model yielded an estimate of ~20-17 ka for the HBV tMRCA (95% HPD), which was more consistent in this regard. This suggests that not accounting for the time-dependency of the evolutionary rate may have led to an underestimation of deep divergence times. However, model selection favored the use of a relaxed clock over a TDR model (log BF: 405) (*22*). Taken together, these results point to a scenario in which the MRCA of all HBV strains examined to date existed around the end of the Pleistocene and gave rise to one or several lineages that spread across Eurasia and eventually reached Africa and Oceania, and to another lineage that spread into the Americas with early settlers of this continent.

Our findings challenge the view that current HBV diversity reflects early human dispersals out of Africa. This model is supported, in particular, by the exclusive association of HBV subgenotype C4 with the Aboriginal people of Australia, suggesting that this subgenotype may have been carried by the first settlers of Australia at least ~50 ka (*5*, *20*). Instead, in accordance with previous findings (*14*), our results indicate that all known modern and ancient HBV strains descend from a lineage that began to diversify at a more recent stage of human history, and that subgenotype C4 was introduced in the Australian continent after ~4.5 ka (Fig. 2). Nevertheless, the age of the observed MRCA only represents a lower limit for the earliest presence of HBV in humans. Whether the latter has been preceded by long coevolution, a recent spillover from another animal species, or any intermediate scenario, remains an open question. Other viruses from the Hepadnaviridae family have been recovered from a wide range of vertebrates, but none of them appear to represent an ancestral zoonotic source for the human HBV (*8*).

HBV circulated widely in western Eurasia as early as 10 ka

The retrieval of HBV genomes from around 10 ka in different parts of Europe and Anatolia, indicate that the virus was widespread in western Eurasia at that time (Figs. 1, S1). The oldest HBV strains recovered in Europe form two distinct clades (Figs. 2, S2; table S2): one that was found in three hunter-gatherers (HG) from northwestern Russia, Belgium and Doggerland (Mesolithic 1), and another that was found in a HG from western Russia (Mesolithic 2). These two lineages are placed within the Eurasian branch as sister groups to the modern strains found in non-human primates (NHP) from Southeast Asia and Africa, respectively. The position of NHP HBV lineages within human HBV diversity has been observed in most previous phylogenetic reconstructions and is thought to reflect spillover events from humans to NHPs (*7*, *22*, *28*). The HBV genome reconstructed from an early Anatolian farmer forms a separate lineage recovered at a phylogenetic position intermediate to the two European Mesolithic clades. Between ~9 and 7.5 ka, HBV strains found in HGs from Karelia (northwestern Russia), Sweden, Luxembourg and Sicily all belonged to the Mesolithic 2 clade. Thus, although our data do not allow detailed phylogeographic inference, they suggest that, during the early Holocene, HBV strains could spread over large parts of western Eurasia within a few thousand years. This is consistent with evidence of genetic connections between Europe and the Near East that predate the Neolithic transition (*29*, *30*), and with the observed genetic cline from Western to Eastern HGs (*31*). Our results further highlight that Mesolithic populations likely formed a network through which pathogens could spread.

It has been suggested that most human-adapted pathogens emerged after the Neolithic transition in association with sedentary lifestyles, increased contact with domesticated animals, and higher population densities, a phenomenon sometimes referred to as the “first

epidemiological transition" (*32*–*34*). Our finding of widespread HBV in HG populations indicates that HBV was present prior to the advent of agriculture and animal husbandry in different parts of the world. Today, HBV rarely causes lethal fulminant hepatitis, but rather asymptomatic infections that may evolve into chronic forms, sometimes developing into liver complications and possible liver failure after decades of infection (*1*, *2*). Although it is difficult to extrapolate from present-day medical studies what the clinical impact of a pathogen would have been in the past, given different diets, disease burdens, and life expectancies, the virus has likely exhibited similar pathophysiological features. Consequently, our findings are consistent with the view that, although small HG communities could not sustain highly epidemic "crowd" diseases, they could maintain chronic infectious agents (*35*, *36*).

A replacement of HBV diversity occurred with the Neolithic transition in Europe

Our data show that HBV remained widespread in Europe after the Neolithic transition (8-7 ka), with numerous strains recovered from early European farmers (EEF) across the continent (Figs. 3, S1; Data S1). Remarkably, all of these strains belong to a single HBV lineage that does not descend from previously observed Mesolithic strains (Figs. 2, 3, S2). We refer to this HBV lineage as the Western-Eurasian Neolithic-to-Bronze-Age (WENBA) lineage. This transition is also observable at a micro-scale in Grotta dell'Uzzo (Sicily), where HBV strains recovered from Neolithic individuals are unrelated to a Late Mesolithic strain identified at the same site (figs. S1, S2). This suggests that the HBV strains observed in EEFs were not acquired from local HGs in different areas, but were rather disseminated by EEFs themselves. While EEFs ultimately derived from early agricultural populations in the Near East (*37*, *38*), the strain we retrieved from an Anatolian farmer dated to ~10 ka was not ancestral to the WENBA lineage (Fig. 2). Therefore, even if EEFs were indeed key in disseminating WENBA strains, whether this lineage originated in Near Eastern centers of early agriculture or in another location along EEF's expansion routes remains to be determined. Furthermore, given the current sample availability for this period, a scenario in which the WENBA lineage would have originated and disseminated among European HGs shortly before the Neolithic transition cannot be completely excluded.

Later, we find WENBA HBV strains in two HGs from transitional Neolithic contexts in western Russia dated to ~7.2 and ~6.4 ka (JAZ001 and MUR007), as well as on both sides of the Greater Caucasus Mountain range and in Anatolia as early as ~5.6 ka (fig. S1). In general, phylogenetic relationships among HBV sublineages within the WENBA clade do not exhibit a strong geographical structure (fig. S2), nor do they seem to reflect the material culture or genetic profile of the individuals in which they were found (fig. S6). Furthermore, our phylodynamic reconstruction indicates that, after an initial growth phase, the transmission of WENBA HBV reached an equilibrium from ~7.5 to ~3.5 ka (fig. S7). Overall, this suggests that HBV strains disseminated by EEFs quickly spread throughout much of western Eurasia beyond the limits of the European agricultural expansion, where they became endemic and continued to circulate widely across different populations, for several thousand years. In particular, we do not observe significant changes in the HBV genetic landscape associated with the expansion of steppe-related ancestry that dramatically altered the genetic profile of Europeans from ~5 ka onward (*37*) (Figs. 2, S2; Data S1). Sexual and perinatal transmission have likely always been the major mechanisms of HBV infection in humans, but cultural practices involving contact with blood [e.g., tattooing (*39*)] or non-sexual violent interactions (*40*) could also have played a role in the spread of the virus in the past. In

general, our findings attest to a degree of interconnectivity among prehistoric populations of different origins, subsistence modes, and cultures that allowed for the dissemination of directly-transmitted pathogens.

The 2nd millennium BCE collapse of WENBA HBV

Following the Early Neolithic (8-7 ka), the WENBA HBV lineage prevailed in most parts of western Eurasia for more than 4,000 years (Fig. 3). However, the latest occurrence of a WENBA strain in our dataset is dated to ~3.3 ka, after which this lineage is no longer observed (figs. S1, S2). In contrast, genotype A, which we first observe at the eastern edge of Europe and in the Near East between ~5 and ~3.5 ka, still appears after ~2.5 ka, by which time it had reached the Carpathian Basin in central Europe. Around the same date, we first observe genotype D in two individuals from the Italian Alps, as well as in various locations in the western steppe, before prevailing in large parts of Europe during the Medieval period. Thus, it seems that as most WENBA HBV lineages disappeared by the end of the 2nd millennium BCE, genotypes A and D subsequently spread from eastern reservoirs to eventually reach western regions that had previously only harbored WENBA strains (*22*). The second half of the 2nd millennium BCE bears witness to major cultural shifts in the archaeological record in western Eurasia, including the sudden disappearance of tell settlements in the Carpathian Basin (*41*), the expansion of the Urnfield culture and the increase of military conflicts in large parts of Europe (*42–45*), the breakdown of the Terramare culture in northern Italy (*46*), and the so-called Late Bronze Age collapse of most state societies in the eastern Mediterranean region and Near East (*47*, *48*). Some of these societal transformations could have been triggered by underlying phenomena such as climatic events (*49*) or the spread of epidemic diseases (*50*), and were likely associated with significant shifts in population densities, trans-regional networks, and modes and scales of human mobility. The observed decline of WENBA HBV diversity, as well as our phylodynamic reconstruction (fig. S7), further point to important changes in epidemiological dynamics over large parts of western Eurasia during this period. However, while our data suggests that new lineages disseminated across Europe only later on, the lack of observations around 3 ka (Fig. 3) could reflect sampling biases related to the widespread adoption of cremation practices around that time (*42–44*), rather than a decrease of HBV prevalence. Searching for the virus in a large number of systematically dated samples across this period could help to better characterize the process that ultimately led to the renewal of western Eurasian HBV diversity after the end of the 2nd millennium BCE.

Recent re-emergence of the WENBA HBV lineage

The majority of HBV strains circulating in western Eurasia today belong to genotypes A and D (*3*, *4*), thus only reflecting a relatively recent part of the phylogeographic history of this virus. However, our results show that despite the seemingly complete disappearance of WENBA HBV strains around the end of the 2nd millennium BCE, one lineage descending from this clade has, in fact, persisted to the present. The latter gave rise to a group of modern strains classified as genotype G (Figs. 2, S2), a rare, recently described genotype for which the biology is poorly understood (*51*). First discovered in patients from France and the United States, genotype G was later found in other parts of Europe, the Americas, and in Asia, making its geographic origin unclear (*52*). Despite its wide distribution, genotype G exhibits remarkably low genetic diversity (*53*), suggesting a recent re-emergence after thousands of years of low-level persistence. Furthermore, genotype G has mostly been

found in HIV-positive patients, and phylodynamic patterns have pointed to a sharp increase of its dissemination co-occurring with the HIV pandemic, possibly associated with highly sexually active groups and injection drug users (*52*).

Genotype G has sometimes been referred to as “aberrant” due to its unique genomic features: a 36-nt insertion near the 5’ end of the core gene and two nonsense mutations in the pre-core region (*51*, *54*). These changes inhibit production of the immunotolerogen e antigen (HBeAg), which appears essential for the establishment of a persistent HBV infection, and alter the structure of the HBV core protein, which may impair packaging and replication of the viral genetic material (*54*, *55*). This likely explains why, in the vast majority of cases, genotype G occurs in co-infections with other HBV genotypes, which can provide the HBeAg and core protein production functions lacking in genotype G (*54*–*56*). We identified similar insertions and stop codons in 14 ancient HBV genomes ranging in age between ~7 and 3.5 ka, which form the WENBA subclade from which genotype G descends (fig. S8). Additionally, most of these ancient genomes were found in individuals showing signs of infections with several HBV variants [fig. S8; Data S2; (*22*)]. In fact, cases of mixed infection were exclusively found in individuals carrying WENBA HBV strains, among which they were very frequent (22/83 individuals, likely underestimating the true frequency). In all cases, both major and minor strains appeared to belong to the WENBA lineage, and sequencing data were partially supporting a ~40-bp insertion at the 5’ end of the core gene (table S3; Data S1).

Therefore, while genotype G is considered rare today, it seems that the co-transmission of its ancestral form together with another HBeAg+ WENBA strain was a common epidemiological feature of HBV between ~7.5 and 3.5 ka. On the other hand, it may appear surprising that this functionally-limited variant specifically persisted until today while the rest of the WENBA HBV diversity seemingly went extinct. Virologic studies indicate that genotype G tends to outcompete HBeAg-producing strains during late HBV infection stages following anti-HBeAg seroconversion (*56*–*58*). It is tempting to speculate a link between these short-term selection patterns and the survival of this lineage over thousands of years, but the latter might also be related to less deterministic factors. One of the closest Bronze Age ancestors of genotype G was recovered at the archaeological site of Shagara in the eastern European forest zone (SGR003; figs. S1, S2), a location where the nowadays-widespread genotype A was already circulating (SGR004). Of note, genotype A is the most common genotype found with genotype G in mixed infections today (*55*, *57*). The discovery of ancestral forms of both genotypes at the same archaeological site, albeit from different individuals and time periods, may indicate that this viral association had already formed during prehistory in eastern Europe.

Conclusions

This study demonstrates the value of large-scale paleogenomic analyses for studying the phylogeographic history of HBV. DNA-enrichment allowed us to reconstruct large proportions of over one hundred ancient HBV genomes from a variety of skeletal tissues, opening important possibilities for future paleovirologic studies. We show that HBV was already widely present in humans during the early Holocene, and that its phylogeographic history reflects several well-known human migrations and demographic events, including the expansion of First American populations in the Americas and the Neolithic transition in Europe, but not others, such as later Bronze Age steppe ancestry expansions. Furthermore, our results reveal patterns that were not expected on the basis of human genetic and

archaeological data alone, such as the near complete renewal of western Eurasian HBV diversity around the end of the 2nd millennium BCE. These findings highlight that the reconstruction of ancient viral diversity has great potential to contribute to our understanding of human history.

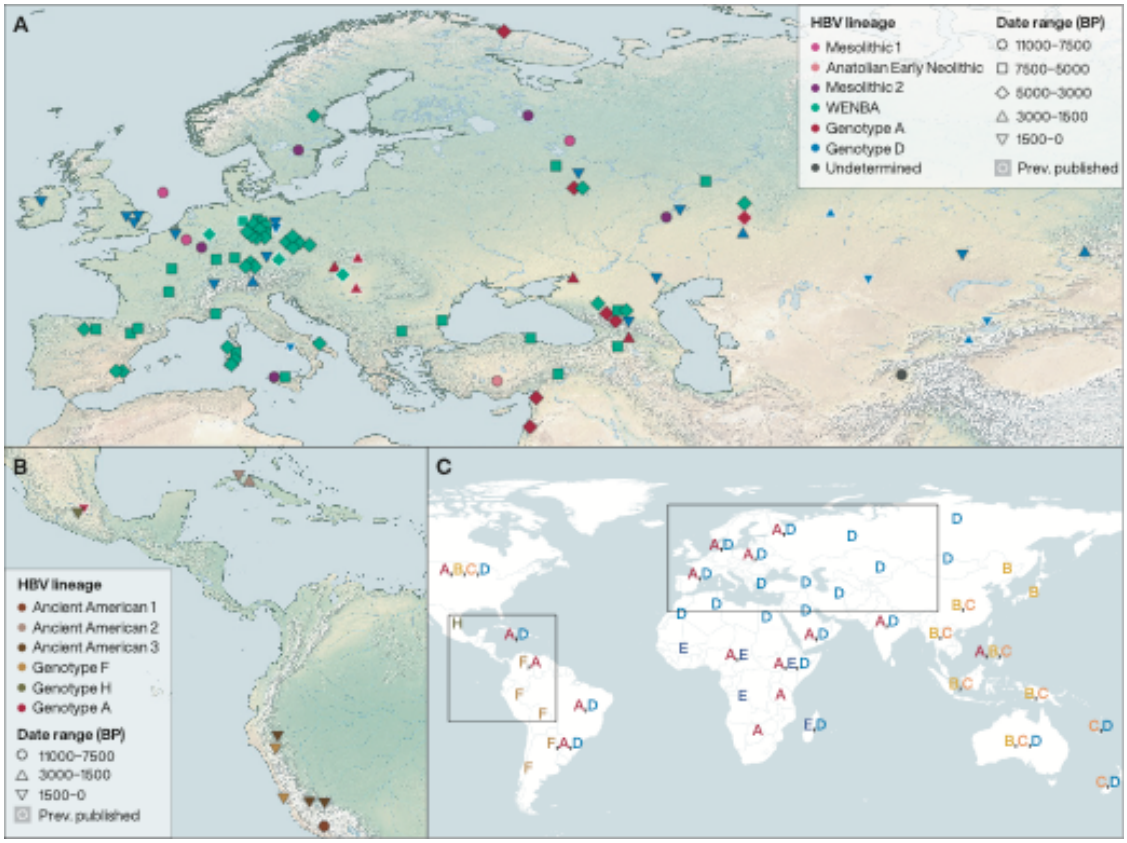

Fig. 1: Geographic location, time period, and lineage of ancient HBV genomes from (**A**) Eurasia and (**B**) the Americas. (**C**) Main distribution of present-day HBV genotypes [adapted from *(4), (14)*].

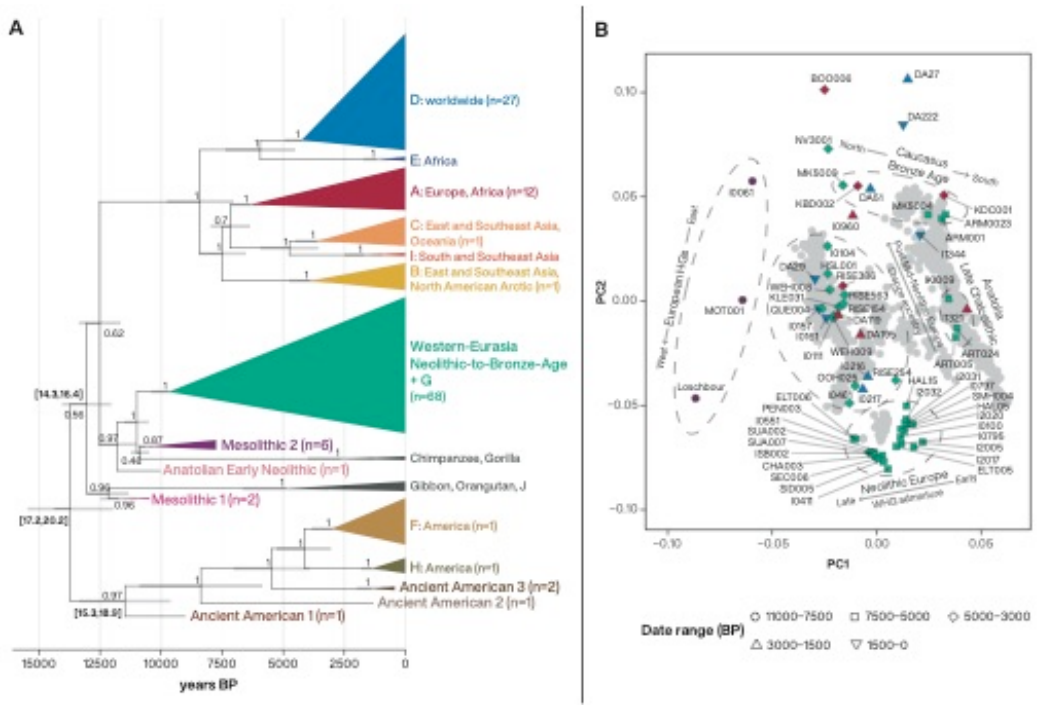

Fig. 2. HBV phylogeny and genetic profile of infected individuals. (**A**)Time-calibrated phylogenetic tree of HBV obtained using a skyline coalescent tree prior and a lognormal relaxed clock. Main clades were collapsed and annotated with their typical present-day geographic location and the number of ancient genomes they contain. Posterior node supports and date estimates (grey bars, indicating 95%HPD) are reported. Additional time-intervals written on deep nodes are 95% HPD estimates obtained with a time-dependent rate model. (**B**) Principal components analysis plot of modern and ancient western Eurasians summarizing the genetic variation of a subset of individuals for which human genetic data was available. Individuals are colored according to the lineage of the HBV strain they carried, as in the tree.

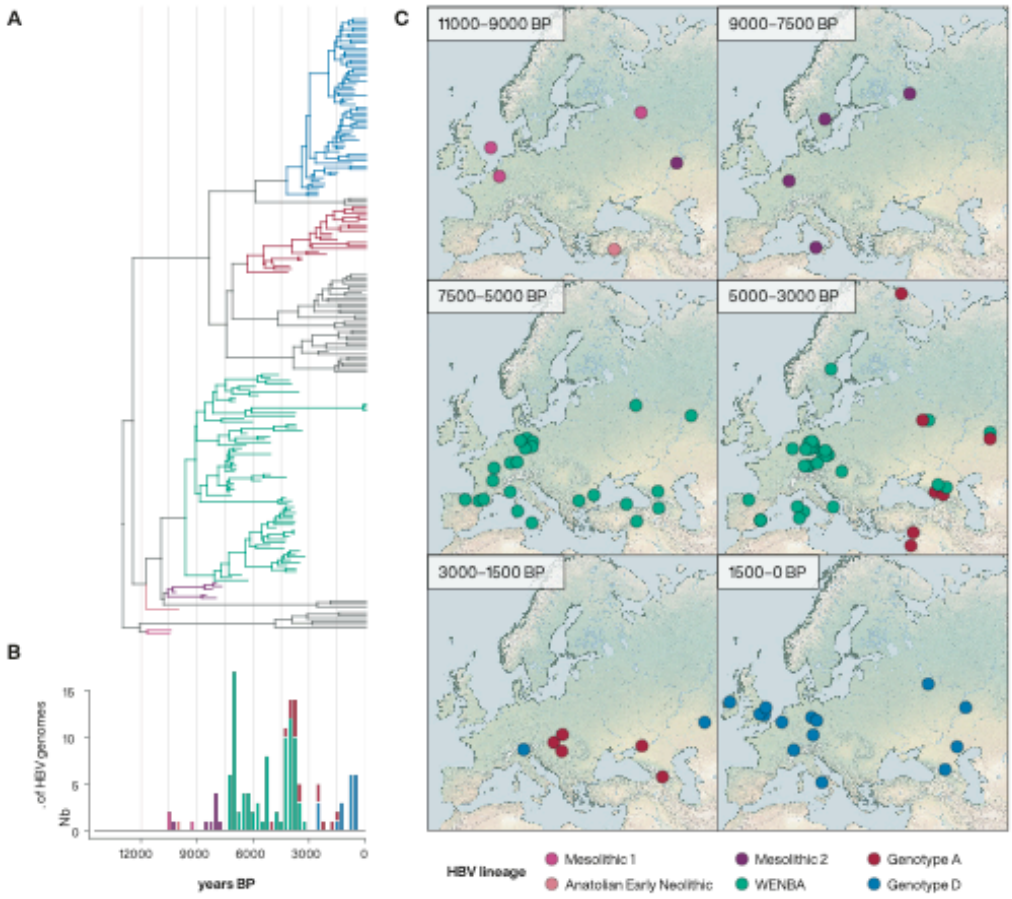

Fig. 3. Spatiotemporal distribution of ancient western Eurasian HBV strains. (**A**) Time-calibrated phylogenetic tree (Eurasian branch). Lineages containing ancient HBV genomes are colored. (**B**) Histogram showing the number of recovered ancient HBV genomes belonging to each lineage through time. (**C**) Geographic distribution of ancient HBV genomes within different time-periods, colored by lineage.

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Supplementary Materials Contain:

- Materials and Methods
- Supplementary Text
- Figs. S1 to S10
- Tables S1 to S5
- Data S1 to S4
- References

Supplementary Materials for Manuscript C

Ten millennia of hepatitis B virus evolution

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This PDF file includes:

Materials and Methods
Supplementary Text
Figs. S1 to S10
Tables S1 to S5
Captions for Data S1 to S4

Other Supplementary Materials (burnt onto CD which accompanies this thesis) for this manuscript includes the following:

Data S1 to S4

Materials and Methods

HBV Detection in ancient individuals

Shotgun sequencing data obtained from ancient human remains in the context of projects conducted at the Max Planck Institute for the Science of Human History were screened for traces of HBV DNA using *MALT ver. 0.3.8* (*59*), with a reference dataset representative of known modern and ancient HBV diversity as well as other orthohepadnaviruses (Supplementary Methods). DNA vector sequences containing Woodchuck Hepatitis Virus or HBV Posttranscriptional Regulatory Elements were also included in the reference dataset to avoid false positive detection due to these potential laboratory contaminants. Reads mapping specifically to HBV were extracted from the *MALT* results (rma6 files) for further examination using *Maltextract* (part of the *HOPS* pipeline (*60*)) with a minimum percentage identity (–minPI) of 80, a top percent value (–top) of 0.05 and all other parameters set to default.

DNA capture and sequencing

Approximately 4% (325/7918) of screened datasets showed putative traces of HBV DNA. HBV-DNA enrichment using in-solution capture was performed for candidate DNA libraries prepared from 136 Eurasian and American individuals dated between ~10.5 and 0.4 ka. (Data S1). Among individuals for which the age and sex information was available, 67% were adults (80/119), and 56% (67/120) were males. For some of the individuals, several libraries were included, resulting in a total of 154 libraries (Data S1). 104 of these were prepared from teeth, while 50 were prepared from bones, including 32 from the petrous bone. HBV probe sequences were designed based on the reference dataset used for screening with a 1-bp tiling, a length of 52 bp and an additional 8-bp linker sequence (CACTGCGG), as previously described (*12*, *61*). Low-complexity and duplicated probes were removed, resulting in 221,190 unique probe sequences. The set was quadrupled to fill the feature space of an Agilent SureSelect DNA Capture Array and turned into an in-solution DNA capture library (*61*). Candidate libraries were reamplified with IS5/IS6 primers and Herculase II Fusion DNA Polymerase to reach a concentration of ~400 ng/µL as measured on a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific Inc.; Waltham, MA). Capture was performed on 5.25 µL of each library. After two consecutive rounds of enrichment, a reconditioning PCR was made to eliminate heteroduplexes originating from mixed-template PCR products. Enriched libraries were then pooled equimolarly (10 mM final concentration), and prepared for shotgun sequencing on an Illumina HiSeq 4000 Systems platform (Illumina, Inc., San Diego, CA) with 2x75 paired-end cycles.

Sequencing data preprocessing

Raw sequencing reads were assigned to corresponding libraries based on sequenced P7 and P5 indices (allowing for one mismatch per index). Adapter trimming and paired-read merging were then performed using *AdapterRemoval* 2.3.0 (*62*). Fragments smaller than 30 bp or containing more than three unresolved nucleotides ('N') were excluded. In order to limit the impact of inter-sample contamination due to index-jumping (*63*), we filtered sequencing reads based on their copy number similarly to Esling *et al.* (*64*). In brief, the rationale was that reads originating from authentic DNA molecules amplified in a given library should exhibit higher duplication levels than spurious reads arising from molecular and sequencing artefacts. For each library, we recovered reads carrying one of the two library indices, but for which the second index resulted in a combination that wasn't used in any of the sequenced libraries (i.e. necessarily resulting from index jumping). Reads assigned to the given library were kept only if their copy number was significantly higher than those recovered with unused index combinations, as assessed by the modified Thompson τ-test for

outlier detection (*64*). After DNA damage assessment, one bp was clipped from both ends of reads. Resulting files were then combined with HBV reads recovered from shotgun sequencing of the same libraries, and merged for each individual.

HBV genome assembly

Due to evolutionary divergence, ancient HBV genomes may significantly differ from modern ones. Because this could hamper the reconstruction of ancient HBV genomes through a reference-mapping strategy, we first performed *de novo* assembly of the reads with Novoplasty (*65*), using a genome range of 3000 to 3500 bp, a k-mer length of 24 and the single-end read option (reads were already merged at this step of the analysis). When a complete circularized assembly could not be obtained, we mapped reads against the HBV reference genome (GenBank accession: NC_003977) within *Geneious* 9.1.8., allowing for 15% mismatches and 10% gaps. We used a fine tuning with 5 mapping iterations against the temporary consensus, which allows recovering highly divergent regions. Alignments were then visually inspected and amended if necessary. In particular, we checked for the consistency of reading frames within each gene and for alignment issues around large indels. Preliminary consensus sequences were produced from these alignments using a 1x coverage threshold and a 50% majority rule.

Resulting *de novo* assemblies and preliminary consensus sequences were then used as references for corresponding sequencing datasets within the *EAGER* pipeline (*66*), in order to produce final conservative consensus sequences as well as coverage and damage statistics: reads were mapped using the CircularMapper option with a mismatch parameter (-n) of 0.01, a quality filtering (-q) of 30 and an elongation factor of 500. Mapped reads were deduplicated with *Markduplicates* (part of the *Picard* tools; http://broadinstitute.github.io/picard/) and realigned around indels using the *GATK toolkit* (*67*). Resulting binary alignment map (BAM) files were then used to produce tables detailing the number and nucleotide composition of reads covering each genomic position with *pysamstats* (https://github.com/alimanfoo/pysamstats). Finally, we called consensus sequences for each genome using a 3x coverage threshold with a 90% majority rule. The same procedure was used to assemble an HBV genome recovered from the Loschbour individual's shotgun sequencing data (*38*), and to reanalyse the data previously published by Krause-Kyora *et al.* (*13*).

In order to assess the effect of the skeletal element (bone vs. tooth) and library protocol (single-stranded vs. double-stranded) on the success of HBV genome reconstruction, we used a linear mixed-effect model as implemented in the *R* package *lme4* (*68*). Mean HBV coverage was used as the response variable after determining the optimal Box-Cox transformation. Because in some cases, several libraries were sequenced from the same individual, a full interaction mixed effect model with the individual as a random effect was then fitted. Starting with the full-interaction model, backward model-selection was performed using ANOVA to find the simplest model which still adequately described the data. Marginal means were estimated to assess the relative effect of each level within predictive variables, and the significance of observed differences, while still accounting for random effects. The analysis was repeated using a skeletal element variable in which the petrous bone was distinguished from other bones. Finally, we used the same approach to assess whether a single-stranded library protocol would reduce the difference of coverage obtained between the double and single-stranded regions of the HBV genome (fig. S9B). In that case, the ratio of mean coverage obtained in these two regions for each genome was used as the response variable. Although its precise location along the genome may vary, the single-stranded region was considered to be located between position 750 and 1600 for this analysis (*69*). Genomes for which the single-stranded region had a coverage of 0 were excluded from the analysis.

DNA damage assessment

Deamination profiles typical of ancient DNA damage were investigated. For some individuals, we captured several DNA libraries that had not necessarily undergone the same uracil-DNA-glycosylase (UDG) treatment (*70*). Damage assessment was therefore done separately for each library. Untrimmed reads were input in *EAGER* as described above, and the pipeline was complemented with DNA damage assessment using *mapDamage* (*71*). The level of damage on human DNA was assessed in each library using the same pipeline with the GRCh37/hg19 Human reference genome. The correlation between damage on HBV and human DNA was assessed using a linear regression of the C to T substitution frequency at the 5' end of human and HBV-mapping reads respectively (excluding UDG-full libraries and libraries from which less than 100 reads mapping to HBV were found after filtering and deduplication; fig. S9F).

Identification of mixed HBV infections

To assess the presence of mixed HBV infections in some individuals, we looked for heterozygous-like signals along the HBV genome sequence in sequencing read alignments (Data S2). Our rationale was that in case of a mixed infection, one should observe more frequent heterozygous-like positions than expected with molecular and sequencing artifacts only. For each sample, we computed the relative support of the two major variants at each HBV genomic position, for which we also derived a 95% confidence interval using Wilson's method (as implemented in the *R* package *binom*). We then defined heterozygous-like positions as positions being covered at least 10x and for which the support of both major and second major variants was significantly below 90% and above 10% respectively. Cases where the major variant was C or G and the second variant was T or A, respectively, were excluded, in order to discard any heterozygous-like signal possibly due to DNA damage. The baseline probability of a position to be detected as heterozygous-like was estimated as the overall fraction of heterozygous-like positions over positions covered >10x in the complete dataset. A given sample was considered as an outlier and indicative of a mixed infection if it contained a number of heterozygous-like positions that exceeded the 95% quantile of a binomial distribution parameterized with the above-mentioned baseline probability and the number of positions covered >10x in the given sample as number of trials. The results were identical when using the 99% quantile instead. Additionally, we visually inspected read alignments around the 5' end of the C gene. Cases where only a subset of the reads carried a ~40-bp-long insertion (similar to the one found in genotype G) were also considered as evidence of mixed infections. The consensus sequence obtained from each sample showing signs of mixed HBV infections was expected to represent the genome sequence of the most abundant strain. We also tried to estimate to which lineage the minor strain belonged using the *EPA-ng* algorithm [(*72*); see below]

Genomic dataset and sequence alignment

For phylogenetic analyses, we gathered a set of modern HBV genomes encompassing the currently described diversity of the virus (Supplementary Methods) to which we added previously published ancient HBV genomes (*11–16*) as well as the genomes generated in this study for which at least 50% of the sequence was resolved (105/137). Sequences were aligned using *MAFFT* v7.475 (*73*) with the iterative refinement method for global alignments. The resulting alignment was inspected using *Geneious* and corrected around large indels when necessary. Highly divergent and potentially misaligned regions, as well as regions containing indels, were masked from the alignment using *Gblocks* (*74*) with default

parameters. An additional stretch of 9 nucleotides flanking the location of large insertions in the preS1 region (pos. 2990-2998) appeared potentially misaligned and was masked as well.

Temporal signal assessment and phylogenetic analyses

The HBV genome alignment was used to construct a phylogenetic tree with *RAxML* v. 8.2.12 (*75*), using the GTRCAT substitution model and the rapid bootstrap algorithm with the autoMRE bootstopping criterion. The resulting ML tree was midpoint-rooted, and the temporal signal of the genomic dataset was assessed using a linear regression of root-to-tip genetic distances (measured in substitutions/site) against genome sampling dates (*76*). Root-to-tip regression indicated that our dataset exhibited a strong temporal structure (fig S3), which was further confirmed by a formal Bayesian model comparison [(*77*); see below].

We therefore performed a time-calibrated phylogenetic analysis using *BEAST2* (*78*). Genome ages were used as calibration points (using the midrange of C14 or archeological dating, and 0 for modern genomes). In order to choose the most appropriate tree prior and clock model, we performed model selection using path sampling (*79*) as implemented in the *model-selection* package. We compared constant coalescent, exponential coalescent, Bayesian skyline coalescent (*80*) (with either 5 or 10 population size groups), and birth-death skyline (*81*) tree priors, each of which were combined with either a strict or a relaxed lognormal clock model (*82*). For coalescent tree priors, the upper bound of the population size was set to 38M (default value increased by two orders of magnitude). For the birth-death skyline model, we allowed 5 shifts of the reproductive number, for which we used a lognormal prior with mean=0 and sd=0.5. We considered a constant rate to become non-infectious with an exponential prior with mean=0.01, and a constant sampling proportion with a uniform prior between 0 and 1. Additionally, we parameterized the model using a sampling probability ρ at time 0 with a uniform prior between 0 and 1. For each of these models, we used a GTR+GAMMA+I substitution model with four gamma categories. A uniform distribution between 10^{-9} and 10^{-3} subst.site^{-1}.year^{-1} was used as a prior for the mean clock rate, based on the range of previous estimates (*10*, *14*). The weights of tree-related operators were increased three-fold in order to improve mixing of the tree topology. All other settings were left at their default values (*BEAUTi* v. 2.5.2). Path sampling was run for each of these models using 16 steps of 80M MCMC iterations and 50% burn-in. Resulting marginal likelihood estimates were used to compare the fit of each model. We then used path sampling in the same way to perform Bayesian evaluation of the temporal signal (*77*). The previously selected model was modified to drop the time-calibration information: tip dates were not used and the clock rate was fixed to 1. The use of authentic genome dates yielded a better model fit, indicating significant temporal signal (log Bayes Factor: 338).

The selected model was used for phylogenetic inference in *BEAST2*. MCMC sampling was performed using 500M iterations including 10% burn-in, or more when necessary. We assessed convergence by ensuring that effective sample size was above 200 for each parameter. A maximum clade credibility (MCC) tree was derived from the posterior tree distribution using *TreeAnnotator* (*83*). We then explored plausible alternative topologies for two deep nodes which had relatively low support in the resulting MCC tree: the Eurasian branch and the clade corresponding to the Eurasian branch with the exclusion of the Mesolithic 1 and Southeast Asian NHP clades. We retrieved MCC trees conditioned on the absence of these respective clades (i.e. based on a tree sample in which all trees containing these clades were filtered out). We then used the full posterior tree sample to compute node posterior supports on these alternative topologies.

In order to evaluate the robustness of our results with respect to different aspects of the dataset and model assumptions, we performed a series of sensitivity analyses. First, because genetic recombination might interfere with phylogenetic inference, we performed an analysis

using an alignment in which recombining regions identified with *RDP4* (*84*) were masked from recombinant genomes. Second, because mixed HBV infections might result in genome assembly issues such as the reconstruction of artificial hybrids, we performed an analysis excluding all ancient genomes recovered from individuals in which mixed HBV infections were detected. Lastly, because it is frequently observed that evolutionary rates may vary depending on the considered time-scale (*85*), we performed an analysis using a time-dependent-rate model as implemented in BEAST v.1.10.4 (*86*, *87*). We used a five-epoch model with transitions every 2.000 years, and the midpoint of each epoch as a reference point to compute the epoch's rate (taking 9 ka for the last epoch). We used Normal priors with mean=-11.5 and 0 and sd=5 and 5 for the intercept and slope coefficients of the log-linear relationship between the substitution rate and time, respectively (corresponding to an expected rate of ~10^{-5} subst.site^{-1}.year^{-1} at time 0 and no prior assumption on the direction of the relationship). Other model specifications were identical to the main analysis. Additionally, we assess the performance of this model compared to previously tested clock models using path sampling as previously described using BEAST v.1 (*88*).

Genomic characteristic and genotyping of ancient HBV strains

Examination of inter-strain genetic distances highlighted the difficulty to consistently classify ancient strains into genotypes using the convention used for modern ones (*i.e.* an average genetic distance threshold of 7.5%). Indeed, some ancient genomes showed less than 7.5% divergence with modern strains from several genotypes, and the use of this threshold would categorize ancient strains into groups that were not necessarily recovered as monophyletic (table S4; supplementary text). Therefore, ancient strains grouped monophyletically and showing less than 7.5% genomic divergence with modern strains from a given genotype were classified as such. Otherwise, we used a classification scheme based on well-supported phylogenetic clades that seem to have a consistent spatiotemporal distribution: Mesolithic lineages 1 and 2, Anatolian Early Neolithic lineage, Western-Eurasian Neolithic-to-Bronze-Age lineage, Ancient American lineages 1, 2 and 3. Finally, we inferred the serotype of ancient strains based on their surface protein sequence (*89*), assessed their HBeAg status based on their pre-core region sequence, and looked for previously described or unknown genomic indels (*3*) by visually inspecting the sequence alignment (fig. S8, Data S1).

Phylodynamic analysis

We explored the transmission dynamics of WENBA HBV strains in Eurasia using a birth-death skyline model as implemented in BEAST2 (*81*) with a dataset consisting of only WENBA strains. When several genomes were recovered from the same site and appeared closely related based on the initial phylogenetic analysis, only one genome was kept (the most resolved one), in order to reduce sampling bias (see Supplementary Text for the list of excluded genomes). The dataset was complemented with 35 sequences of modern genotype G genomes taken from a previous phylodynamic analysis [[(*90*); see Supplementary Text for a list of accessions]. Sequences were aligned and highly divergent regions were masked as previously described. Because of the restricted dataset employed for this analysis, a strict clock model was used, with a uniform prior between 10^{-9} and 10^{-3}.subst.site^{-1}.years^{-1} for the substitution rate. Based on the assumption that the majority of HBV infections are long-lasting chronic infections, we used a constant rate to become non-infectious with a lognormal prior with mean=0.05 (in real space) and sd=0.1, corresponding to a 95% percentile interval for the average duration of infectivity between ~15 and ~25 years. For the diversification/extinction ratio (reproductive number) and the sampling proportion, we allowed three time shifts (i.e. four parameter values), and (i) a lognormal prior with mean=3

(in real space) and sd=1 for the diversification/extinction ratio and (ii) a uniform distribution between 0 and 1 for the sampling proportion. Time shifts were estimated within three equally-spaced time windows between 0 and 9 ka (allowing a fourth shift did not appear to yield additional information). The posterior distribution of time shifts and piecewise-constant diversification/extinction ratios were used to derive the posterior distribution of the diversification/extinction ratio through time using a grid of 100 years.

Lineage assignment of low-coverage genomes

We used the *EPA-ng* algorithm (*72*) as described previously (*91*) to perform phylogenetic placement of low-coverage HBV genomes that were not included in phylogenetic analyses. Consensus sequences were obtained for low-coverage genomes using the previously described approach modified with a 1x coverage threshold. Resulting sequences were aligned with the high-coverage-genome alignment (which was used for phylogenetic analyses) using *MAFFT* with the –add and –keeplength options to preserve the original alignment structure. The resulting alignment was corrected around large indels when necessary, and potentially misaligned regions were masked as previously described. The high-coverage-genome alignment was used as a reference alignment, and the low-coverage-genome alignment was used as a query for *EPA-ng*. The reference tree and substitution model specifications were taken from the previously conducted ML phylogenetic analysis. The taxonomic assignment method of *Gappa* (*92*) was used to assign genotypes or previously defined ancient HBV lineages to each low-coverage genome. We used a taxonomic reference file in which all reference sequences (highly-covered genomes) were associated with a taxonomic path consisting of "Hepatitis B virus" followed by the corresponding genotype or ancient lineage (i.e. we did not allow subgenotype nor intermediate level assignments). For each low-coverage genome, the most supported assignment was identified using likelihood weight ratios (LWRs).

Lineage assignment of minor strains within mixed infections

For samples from which mixed infections where identified, we tried to infer the lineage to which the minor strain belonged based on identified genomic polymorphisms. We used the python package *pysam* (https://github.com/pysam-developers/pysam) to create new BAM files containing only the sequencing reads carrying minor variants at identified heterozygous-like positions, with the additional condition that the variant support was significantly below 40%. This was to avoid selecting mixtures of reads corresponding to several strains (in case those were found in even proportions), and left 12 samples for which such reads could be retrieved and analyzed. Consensus sequences were obtained from these alignments using a 1x coverage threshold, and used to perform phylogenetic placement with *EPA-ng* as previously described (table S3).

Detection of recombinant genomes

RDP4 (*84*) was used to detect signals of genetic recombination in our dataset. A complete exploratory search was run on the sequence alignment used for phylogenetic analyses with the seven integrated methods employed by default. Events recovered by all methods were further examined iteratively, starting with the most supported ones. Based on the RDP plot and the topology of local ML trees (constructed with the minor and major parental regions of the alignment), the event was either confirmed or rejected. More specifically, we expected that a group of genomes identified as resulting from the same recombination event should form a monophyletic clade closely related to respective parental genomes in local ML trees. In some cases, swapping parental or recombinant genomes in the identified triplet resulted in more likely scenarios. Examination of local ML trees sometimes

revealed that close relatives of identified recombinant(s) were affected by the same event, in which case they were marked as such. When a recombination event was confirmed, we accepted it and performed a rescan of the dataset. The procedure was repeated until no further unaccepted event was recovered by all methods.

Human population genetics

Human genome-wide data for some of the ancient individuals from which HBV genomes were recovered was taken from the latest Allen Ancient DNA Resource (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data) V44.3 January 20 2021. Principal components analysis (PCA) was conducted using *smartpca* v16000 from the *EIGENSOFT* package (https://github.com/DReichLab/EIG). The principal components were calculated on a set of modern west Eurasian individuals genotyped on ~597000 sites from the Affymetrix Human Origins array (*38*, *93–96*). Ancient individuals were projected onto the resulting principal components (lsqproject: YES) and shrinkmode was used to reduce the effect of modern populations "stretching" of axes (shrinkmode: YES).

Supplementary Text

List of GenBank accessions of HBV genomes used for the screening of shotgun sequencing data and probe design

We included a wide range of HBV genomes encompassing all known modern HBV genotypes as well as ancient genomes and orthohepadnaviruses found in non-human mammals: LT992459, LT992455, LT992454, LT992448, LT992447, LT992444, LT992443, LT992442, LT992441, LT992440, LT992439, LT992438, JN315779, MG585269, AB076679, AB116084, AB453988, AY738142, GQ477499, AY934764, FJ692556, FJ692598, FJ692611, GQ161813, GQ331046, AB073858, AB033555, AB219429, AB219430, AP011089, AB073835, AB287316, AB287318, AB287320, AB287321, DQ463789, DQ463792, AB241117, DQ993686, AB111946, AB112066, AB112472, DQ089767, X75656, X75665, AB048704, AB048705, AF241411, AP011100, AP011102, AP011103, AP011106, AP011108, FJ899792, JN642140, GQ477453, GQ477455, JN642160, JN642163, JN688710, JN688711, GQ922005, HE974378, KJ470893, KJ470896, KJ470898, FJ904430, FJ904436, AB033559, AB048701, AB048702, AB188243, AB210818, AM494716, AY796031, AY902768, DQ315779, X80925, X75657, X75664, AY090458, AB116654, FJ657525, AY090455, AY311369, DQ899144, DQ899146, AB116549, X75663, AF223962, AB166850, AB056513, AB064312, AF405706, AB059660, AB375163, AY090454, AY090457, AB486012, AY330911, AJ131571, AY781180, U46935, AJ131567, AF193863, EU155824, KC790378, KC790377, KC790376, NC_001484, U29144, NC_004107, AF046996, KY703886, MH307930, AF498266.

We also included the three ancient genomes recovered by Krause-Kyora *et al.* (*13*), as well as DNA vector sequences containing Woodchuck Hepatitis or HBV posttranscriptional regulatory elements to avoid false-positive detection due to these potential lab contaminants (those were excluded for probe design):
GQ202119,GQ202120,DQ869006,JN898962,JN898959,KJ796484,AB902850,KJ411917,KJ411912,KJ411915,KJ411918,KJ411916,KJ411919,KJ411911,KT345943,JX006096,JF927991,KX757240,KX757239,KX757255,KX757254,KX757253,KX757252,KX757251,KX757250,KX757249,KX757248,KX757244,KX757247,KX757246,KX757245,X757242,KX757243,KX757241,KX757238,FR822201,JX861384,KM519787,KM519788,JN622008,EF205034,EF205035,KJ697750,KJ697752,KJ697753,KJ697751,HG530137,GQ872121,KC152483,KC152484,KC152485,KC152482,KC152481,JQ086322,KF486506,FJ797421,EF177827,AY468

486,KC262216,GU253315,GU253314,GU253312,GU253313,EU048697,EU048696,EF186079,EU000249,EF186078

List of GenBank accessions of modern HBV genomes used for phylogenetic analyses

Genotype A: AB076679, AB116084, AB453988, AY738142, GQ477499, AY934764, FJ692556, FJ692598, FJ692611, GQ161813, GQ331046

Genotype B: AB073858, AB033555, AB219429, AP011089, AB073835, AB287316, AB287318, AB287320, AB287321, DQ463789, DQ463792, AB241117, DQ993686, FJ899779

Genotype C: AB111946, AB112066, AB112472, DQ089767, X75656, X75665, AB048704, AB048705, AF241411, AP011100, AP011102, AP011103, AP011106, AP011108, AB049609

Genotype D: NC_003977, FJ899792, JN642140, GQ477453, GQ477455, JN642160, JN642163, JN688710, JN688711, GQ922005, HE974378, KJ470893, KJ470896, KJ470898, FJ904430, FJ904436, AB033559, AB048701, AB048702, AB188243, AB210818, AM494716, AY796031, AY902768, DQ315779, X80925, FJ904399, AY721612, AY741797, AB270543, EU594409, AB109476, AB555496, GQ205377

Genotype E: X75657, X75664, HM363593

Genotype F: AY090458, AB116654, FJ657525, AY090455, AY311369, DQ899144, DQ899146, AB116549, X75663, AF223962, AB166850, JN792922

Genotype G: AB056513, AB064312, AF405706

Genotype H: AB059660, AB375163, AY090454, AY090457

Genotype I: AB562463, FJ023669, EU835241

Genotype J: AB486012

Genotype cpz: AY330911, AJ131567, AF498266

Genotype gbn: AJ131571, AY781180, U46935

Genotype oru: AF193863, EU155824

List of genomes used for the phylodynamic analysis

WENBA HBV strains included in the main phylogenetic analysis were used for the phylodynamic analysis. When several genomes were recovered from the same site and appeared closely related based on the initial phylogenetic analysis, only one genome was kept (the most resolved one), in order to reduce sampling bias. Thus, the following genomes were excluded: I0411, I0100, I2005, I2020, I2031, MK5009, MKL025, MIS002, WEH008.

We further included the following modern genotype G genomes from (*52*): AB064310, AB064311, AB064312, AB064313, AB625342, AF405706, AP007264, DQ207798, EF634480, GU563556, GU563559, GU565217, HE981171, HE981172, HE981174, HE981175, HE981176, KF414679, KF767450, KF767451, KF767452, KR230749, KX264500, KY004111, KY004112, AB375170, AB375169, AB375167, AB375166, AB375168, AF160501, AB056513, AB056514, AB056515, JQ707668.

We excluded seven sequences from the original dataset that had length incompatible with functional open reading frames, pointing to assembly issues: EF634481, HE981173, KF779233, KF779235, KF779357, KF779267 and JF439787 (KF779233, KF779235 and KF779357 also contained a highly divergent sequence at the end of the X gene). We also excluded sequence AB625343 which contained a sequence at the beginning of the S gene that appeared similar to that found in genotype H.

Archaeological background

Here we provide information on the archaeological context for some of the samples analyzed in this study (for the rest of the samples, see references in Data S1).

Minino 2, Vologda region, Russia (MN2003): The archaeological complex of Minino comprises artifacts and human remains from two Mesolithic-Early Neolithic cemeteries (Minino I and II), separated by a distance of 230 m, near the lake Kubenskoe in the Vologda region, Russia. **MN2003** (Grave VI/ind. 1) was found in a grave from Minino II, which was initially identified as a single grave by the archaeologists, although it contained remains from two individuals: an adult male (**MN2003**), for which an almost complete skeleton was found, and another adult individual, from which only a few skeletal elements were recovered. This combination of complete and partial skeleton remains within the same grave had never been observed before in the site, but later findings revealed that it was a common feature of Minino (half of the graves in Minino II) (*97*).

Traces of ochre concentrated on the skull or near the pelvic bones, as well as grave goods such as bone and stone knives, flints and pendants, have been found in both cemeteries (*98*). Faunal remains found in both cemeteries revealed some of the animal species that were exploited, which appeared typical of hunter-gatherers (elk, pine marten, water vole, wolf or dog, different fish species; and in the later stages: beaver, fox and bear). Although some burial goods exhibited parallels with Veret'e and Butovo archaeological cultures (*97*), the majority could be attributed to a period spanning from the second part of the Mesolithic to the Early Neolithic, which was confirmed by radiocarbon dating (*99*). Additional information came from bioarchaeological analyses of anthropological material from this site (39 individuals). The inspection of teeth revealed healthy conditions without any signs of caries. Enamel hypoplasia and calculus deposition were present at low levels, and showed the same intensity for both sexes. Further study of additional markers of physiological stress in children confirmed this finding, pointing to a similar status of male and female children in this hunter-gatherer society. Significant craniological variation was noted in the site. A series of male skulls from Minino exhibited clear analogies with synchronous populations of northeastern Europe as well as older Paleolithic hunters. In early development stages of this region, the population exhibited high life expectancy, which decreased during later periods. Isotopic analyses also revealed a changing of diets over time (*100*). All these chronological dynamics of anthropological indicators could reflect the migration processes of the region.

Khvalynsk, Volga region, Russia (KVK001): KVK001 (museum nb. KO128) is a human skull fragment that was discovered in 1927 on the Khoroshensky Island of the Volga River near the town of Khvalynsk, by the local museum staff. It was found in a semi-submerged pebble layer, which also contained abundant remains of Quaternary fauna (*101*). Weinert (*102*), who first published the finding, attributed it to a human from the Paleolithic. Later, Gremyatsky (*103*) concluded that it belonged to a, possibly male, anatomically modern human with some Neanderthal features. As noted by Bader (*104*), the stratigraphy of alluvial deposits in the region is quite uniform and stable. After conducting additional analyzes, the researcher did not rule out that this skull fragment could be associated with the Upper Paleolithic (Aurignac-Solutré). While the pebble layer in which it was found, was attributed to the Mindel-Riss Interglacial, A. P. Ososkov (*105*) considered it possible that the sandstone load of this region was formed during the Late Paleolithic, and the results of radiocarbon dating indicated that the sample belongs to the Early Mesolithic time (OxA-23001, 9045 ±40 uncal BP).

"Tutkaul", Tajikistan. (Individuals TTK002): The site of Tutkaul is located in southern Tajikistan, 70 km southeastwards of Dushanbe in the Dashti-Mazar region. The site was discovered during the archaeological survey of the Nurek dam's flooding area led by A.P. Okladnikov in 1956 (*106*). Excavations were conducted during six field seasons (1963,

1965-1969) as part of a rescue archeological program led by the Tajik Archeological Expedition (*107*). The upper part of the stratigraphy consists of a medieval fortified settlement, followed by levels 1 and 2 which are attributed to the Hissar Neolithic culture. The lowest stratigraphic units (3 and 2a) belong to the Early and Late Epipaleolithic (*108*). At the base of level 2, three burials were identified containing the remains of four individuals: a female adult (burial nb. 1), a subadult (burial nb. 2) and two children (burial nb. 3). The burials were oriented to the SE-NW, the skeletons were in a bent condition on the left side, suggesting that the bodies were tied up before being buried. **TTK002** is the vertebra from a child found in burial nb. 3. An unidentified bone fragment from the same burial was radiocarbon-dated to 8425-8025 cal BP (GV-02104 7450±106).

Grotta dell'Uzzo, Sicily, Italy (UZZ081, UZZ075, UZZ061 and UZZ099): Grotta dell'Uzzo is a large rockshelter on the eastern side of the San Vito lo Capo peninsula in NW Sicily. The discovery of the prehistoric deposits was made in the early 1970s by Giovanni Mannino (*109*), who excavated a trench exposing an in situ Mesolithic sequence. Later in the 1970s, 1980s and in 2004, within a number of trenches both inside and outside the overhang of the rockshelter, evidence was unearthed that occupation at the site occurred from the late Upper Palaeolithic through the Mesolithic and into the Neolithic (*110–116*). The cave was occupied in the early and middle Neolithic, as well as during the Bronze Age and throughout history, being used until recently by shepherds as a stable for sheep.

Grotta dell'Uzzo is a key site for Mediterranean prehistory because its long stratigraphic sequence covers the transition from hunter-gatherer to agro-pastoral economies (*113*, *114*, *116–118*). During the so-called Mesolithic-Neolithic transition, hunter-gatherer producers of Castelnovian-like lithic industries adapted to the climatic and environmental changes that occurred around the 8.2 kyr cal. BP event by exploiting large proportions of marine-based protein, mainly originating from the exploitation of cetaceans (*119*). This phase was followed by the introduction of elements of a farming-based economy by early Neolithic groups that arrived in NW Sicily around 8000 years ago (*113*, *119*).

Another reason for which Grotta dell'Uzzo is noteworthy is that, during the Mesolithic, it was a burial site, where at least 13 individuals were inhumated in 11 burials (*111*, *120*, *121*). Human remains were, however, also found scattered in most of the deposits excavated both within and just outside of the overhang of the rockshelter. A thorough dating programme started by Mannino et al. (*119*) has demonstrated that the loose human skeletal remains date to all the main occupation phases of the cave, including the Mesolithic, Mesolithic-Neolithic transition and Neolithic.

The skeletal remains from Grotta dell'Uzzo that were sampled for this study are attributable to the Late Mesolithic and Neolithic phases of occupation. **UZZ081** is a temporal bone fragment recovered in the superficial layer of Trench U and directly radiocarbon dated to 8520-8180 cal. years BP (MAMS-40721: 7807±26 BP). This calibrated age is corrected for the marine reservoir effect, which in the case of this specimen is affected by around 45±10% marine protein (δ13C: -15.9‰; δ15N: 13.0‰), based on a calculation that takes into account the mixing model elaborated by Mannino et al. (*119*). **UZZ081** is thus contemporary to the so-called Mesolithic-Neolithic transition, which at Grotta dell'Uzzo was characterized by the presence of late Mesolithic hunter-gatherers who produced Castelnovian-like lithic industries (*110*).

UZZ075, UZZ061 and **UZZ099** have all been recovered from deposits that post-date the Mesolithic-Neolithic transition, after which Grotta dell'Uzzo was occupied by farming and agro-pastoral communities. **UZZ075** is a temporal bone fragment recovered in Trench S (stratigraphic spit 5) and is directly dated to 7280-7160 cal. years BP (MAMS-40717: 6310±23 BP), which corresponds to the early Neolithic facies (Stentinello). **UZZ061** is a

phalanx recovered from the superficial layer of Trench H and is directly dated to 6830-6660 cal. years BP (MAMS-48211: 5923±25 BP), which corresponds to a middle Neolithic facies called Stentinello / Trichrome / Serra d'Alto. All the dates reported here have been calibrated using OxCal 4.4 (*122*) and the most recent calibration curve IntCal20 (*123*). **UZZ099** is a temporal bone fragment which was radiocarbon dated to 5989-5907 cal BCE (MAMS-40714 5185 ±31).

Jazikovo (Yazykovo), Volga region, Russia (JAZ001): The remains of an adult woman (**JAZ001**, museum nb. 8619) were excavated at the Yazykovo peatland near the village of Yazykovo, Kashinsky district, Russia, in a cultural layer which was firstly discovered by B.S. Zhukov, A.E. Alikhova and M.V. Voevodsky in 1928 on the banks of the Yakhroma river. The researchers discovered ceramics, the stratigraphic position of which indicated that they were anterior to the Pit-Comb Ware culture (*124*). This pointed to Yazykovo being one of the most ancient Neolithic sites in the Upper Volga region. Today, Yazikovo encompasses three complexes (Yazikovo 1,2 and 3), which are attributed to the Central variant of the Upper-Volga archaeological culture (*125*).

Based on the analysis of ceramics from different sites including Lyalovo, Yazykovo 1 and 2, Nikolo-Perevoz 1 and 2, Kholomonikha, Lipki, Volosovo 1, Malo-Okulovskaya 1, Malo-Borskaya and Balakhninskaya 3, Zhukov identified two variants characterizing the development of Neolithic societies in the Upper Volga region (*124*). The variant presumed to be the oldest one was defined as the "Yazykovo" type: thick-walled pottery with abundant grit in the dough, decorated with parallel carved stripes. The second variant was denominated as the "circular-pit comb complex", due to similarities with the "comb" pottery and particular ornamental features. Further studies have led to a more detailed periodization of the Volga-Oka Neolithic, which divides it into an "early" and "developed" period. In the Upper Volga region, subsistence strategies were maintained across the Mesolithic to Neolithic transition, and the beginning of the Neolithic is solely marked by the appearance of pottery. The Early Neolithic period extends from 7100/7000 to 6100/6000 uncal BP (*126*), and is viewed as a period in which Yazykovo-type ceramics were imported in the region through occasional trade with non-autochthonous individuals, while the local lithic industry remained unchanged (*127–129*). The developed Neolithic period extends from 6100/6000 to 3800/3700 uncal BP (*130*), and is marked by the appearance of, likely locally produced, comb-ornamented ceramics, possibly reflecting population influx. **JAZ001** was radiocarbon dated to 6329 ±21 uncal BP (MAMS-37910), thus placing it at the end of the Early Neolithic period.

The Late Neolithic burial ground of Wenigensömmern, Thuringia, Germany (WSN010 and WSN012): The Late Neolithic (Bell Beaker Culture) burial ground of Wenigensömmern (Sömmerda County, Thuringia, Germany) comprises three small burial groups encompassing 14 graves and 18 individuals (*131*). The cemetery is located 2700 m southwest of the Early Bronze Age cemetery of Leubingen. It is dated to ca. 4300-4100 BP. The anthropological determination was made by Sabine Birkenbeil in 2016. **WSN010** and **WSN012** came from a large grave pit (feature 07/319-1129) that contained remains of four individuals, an undecorated bell beaker, and six flint artifacts. Only their skulls were found,secondarily deposited in the southeast corner of the burial pit, without any directly attributable grave goods. The other two individuals in the grave had been buried in crouched positions.

The Early Bronze Age cemetery of Leubingen, Thuringia, Germany (LEU065): The cemetery of the Early Bronze Age (Únětice Culture) of Leubingen (Sömmerda district, Thuringia, Germany) is located about 750 m southwest of the large Leubingen Tumulus, a

prosperous region at that time (*132*). However, the occupation of the burial ground predated (ca. 4100-3900 BP) the construction of the Tumulus in the classical phase of the Únětice Culture. It is the burial site of at least one settlement located in the direct vicinity. The completely investigated burial site includes 34 graves of the early to middle stages of the Únětice culture containing a total of 47 individuals. Other isolated graves were found in the vicinity. The cemetery is located 2700 m northeast of the Late Neolithic cemetery of Wenigensömmern. **LEU065** was first anthropologically determined by Bärbel Heußner in 2010, and comes from a double grave (feature 09/100-2045), a double burial, which took place with a clear time gap. It is an adult male whose remains laid disarticulated above a regularly buried mature female in a stone cist. No grave goods could be assigned to **LEU065**, while the female individual was buried with culturally specific grave goods: two vessels and a perforated shell disc.

Tell Yunatsite, Bulgaria (I0784 and YUN048): Tell Yunatsite is among the most prominent tells in Bulgaria (110x100x12m). It is situated in Southern Bulgaria, in the western part of the Upper Thracian Plain, 6 km northwest from the city of Pazardzhik, and approximately 1 km southwest of the modern village Yunatsite. The tell developed on a low terrace on the ancient bank of the Topolnitsa River, near to its confluence with the Maritsa River. It is located in a fertile plain bounded by mountains – the Rhodope Mountains to the south, Rila and Ihtimanska Sredna Gora Mountains to the west and Sashtinska Sredna Gora Mountain to the north. The first excavations of the site were carried out by V. Mikov in 1939. Systematic archaeological excavations of the tell's eastern section began in 1976 and continued until present (*133–136*). So far, the excavations have yielded evidence of occupation from the 5th millennium BCE until the 6th century CE – including Chalcolithic, Early Bronze Age, Iron Age and Roman Age occupations, as well as a Medieval cemetery. Long-term occupation was documented in two periods – the Chalcolithic and the Early Bronze Age.

Individuals **I0784** and **YUN048** were found in the latest Chalcolithic settlement (occupation level BI), which provided important information to understand the processes and events leading to the end of the Chalcolithic cultures of the Balkans. It was conquered, burnt and the residents were slaughtered. Some of the bodies (ca. 50 individuals) were discovered buried under the remains of houses destroyed by fire. Some of the human skulls bear signs of injuries. They were probably inflicted by copper (?) battle axes and caused the death of the individuals (*137*). Bones of more than 20 individuals were found scattered between houses. **I0784** (grave 96) is a skeleton found in Building 5 from level BI, sq. K-Л9, belonging to a 40-50 year-old woman (*135*, *137*), and on which two skull traumas were identified. **YUN048** (grave sq. 9) was found in sq. O9, outside of Building 1 (to the north of it), at the "street level" of level BI. The death of this individual was also associated with the destruction of the last Chalcolithic settlement. Genetic sexing suggests that it belonged to a male.

Cueva de las Lechuzas, Spain (CLL005): The cave is located on the eastern slope of the Cabezo de las Cuevas, a small hill in the centre of the Villena basin. The access of the cave was originally about 2 metres wide and gave way to the main room leaning towards the bottom, but it was then destroyed together with part of the cave during quarry exploitation. The archaeologist J.M. Soler identified human remains belonging to at least 18 individuals in the site, including **CLL005** (Arc. ID: Lech 7), an adult male. He also recovered an important set of grave goods consisting of several arrowheads, an axe and a hoe made of polished stone, several pendants made of various seashell species and teeth, and several necklace beads made of fish and stone vertebrae. Several bone punches and ceramic bowls were also found. These materials indicate a use of the cave during the Chalcolithic period (ca 5300-4300 BP).

Peñón de la Zorra, eastern cave, Spain (PLZ001): The cave is located in the eastern slope of the El Morrón mountain range at about 640 m asl. Its entrance was covered by large limestone blocks at the time of its excavation, carried out by J. M. Soler in spring 1964. It is located on the same hill as the town of the same name, where several phases of occupation have been recognised between the Bell-Beaker (Phase 1: ca. 4400 BP) and the Bronze Age (Phases 2-4: ca. 4100-3700 BP). The human remains were completely removed. They correspond to at least six individuals, three adults and three children (4, 6-8 and 10-12 years), both sexes being represented. The adult individuals exhibited high tooth wear, which could be related to their age. Associated with these remains, several copper weapons were recovered: a tongue dagger and a pair of Palmella-type points. A silver earring of 1.3 cm diameter, a necklace made of 14 fish vertebrae and several small bowls were also recovered. Direct dating of some individuals revealed the use of the cave during the Bronze Age, consistent with previous findings, although a previous use during the Bell-Beaker period associated with copper weapons cannot be ruled out. Individual **PLZ001** (Arc. ID: Individuo 1) is an adult female burial which was indirectly dated to the Bronze Age (ca 4200-3500 BP).

Hostivice, Prague-West district, Czech Republic (HOP004): The site was discovered during rescue excavations led by J. Klementová and D. Daněček (Museum Roztoky) in 2007-2008 (*138*). The excavated area covered 10 ha, and more than 1,300 settlement pit features as well as a similar number of post holes were found. The site has been occupied during the Linear and Stroked Pottery cultures, the Funnel Beaker and Řivnáč cultures, Hallstatt (Ha C-D1), Roman Iron Age and Early Middle Ages. More than 33 graves were also uncovered and burials were identified in sunken settlement features from the Funnel Beaker (Baalberge stage), Corded Ware, Bell Beaker and Knovíz cultures (B D – Ha A2), as well as from the La Tène period (Lt B1-C). Anthropological analyses were performed by M. Dobisíková (National Museum). **HOP004** (Grave 22/Feature 691) is a skeleton found in a left-sided, crouched position, with the head towards the south, which was identified as a 40 to 60-year-old female. No grave goods were found with the individual. The individual was dated to the Bell Beaker period based on the archaeological context, and radiocarbon dated to 4400–4100 cal BP (MAMS-38921: 3826±27).

Tell Atchana (Alalakh), Turkey (ALA098 and ALA110): The site is located in the Amuq Valley, Turkey, and belongs geographically to the northernmost stretches of the Levant. It was founded in the terminal Early Bronze Age or earliest Middle Bronze Age (ca. 2200-2000 BCE) and quickly developed into an urban site with palaces, temples, workshop areas and fortification systems during the Middle Bronze Age II (from ca. 1800 BCE on). Textual evidence attests to its significance as the capital of a regional kingdom during the Middle and Late Bronze Age (ca. 1800-1300 BCE), until its nearly complete abandonment around ca. 1300 BCE (*139–146*). Excavations at Tell Atchana were undertaken by Sir Leonard Woolley from 1935-1939 and from 1946-1949 (*147*, *148*). New excavations under direction of K. Aslıhan Yener have taken place since 2003 (*144*, *145*).

Individuals **ALA098** and **ALA110** stem from these new excavations. They were found in the so-called extramural cemetery in Area 3, located right outside the city wall, in which a total of 134 burials were excavated dating to the Middle and Late Bronze Age (*149–151*). Most of them were laid out in simple pits oriented northwest-southeast parallel to the city wall and follow the outline of the slope. The majority of the graves are single burials (as is generally the case in Alalakh) (*151*). Most individuals were buried on their backs or right or left side with their legs flexed on one side (~80%) (*149*). Additionally, eight secondary burials were identified in the extramural cemetery, four of which only consist of two or three

skulls and/or mandibles buried together (*149*). Compared to the intramural burials (on average 3.49 objects per individual), especially those in the Royal Precinct, the burials in the extramural cemetery contain fewer (on average only 1.25 per individual) and less varied grave goods. 58.2% of the graves here did not contain any grave goods, and the others were typically furnished with one to two objects, mainly consisting of pottery and jewelry (*149*, *151*).

Individual **ALA098** (square 45.45, locus 23), was found in a secondary burial of three mandibles, together with a short-neck jar. The sex and age of this individual are unknown. Strontium isotope analyses have shown that this individual likely grew up outside the Amuq Valley (*149*). Individual **ALA110** (square 45.45, locus 48) was found in a single primary burial containing no grave goods. The individual was identified as a 65 to 75-year-old male, and strontium isotope analyses suggested that this individual moved to Alalakh after the formation of the M2, but before the M3 was formed (*149*).

The Middle Bronze Age burials from the tell of Kamid el-Loz, Lebanon (KEL045): The tell of Kamid el-Loz is located in the Beqa'a-plain in Lebanon and is surrounded by the present-day village of the same name (*152*). Today, a cemetery covers the southeastern parts of the tell. Excavations on the tell took place from 1963-1981. They were first conducted by a team from the Universität des Saarlandes (Germany) led by Rolf Hachmann (*153*, *154*) and were renewed in 1997 by Marlies Heinz from the Albert-Ludwigs-Universität Freiburg (Germany) (*152*, *155*). The earliest traces of settlement uncovered date to the 3rd millennium BCE (Early Bronze Age) (*155*). During the Middle Bronze II (ca. 1750-1550 BCE) Kamid el-Loz developed from a village into a city with a palace, a temple, and other administrative buildings, as well as a fortification system and regular living quarters. After the collapse of this first city, inhabitants of either the tell or the surrounding valley used the ruins of two buildings on the northern slopes ("Gebäude I and II") as a cemetery (*152*), from which 24 individuals (7 adults and 17 children) were excavated. The graves were dug directly into the ruins of the buildings, often re-using older walls as grave linings and stones and mud bricks as covering. 11 individuals were buried in simple pits in primary position, four graves were single jar burials of small infants, and nine individuals were buried in groups of three which may represent multiple burials or accidental use of older burials. The positioning of the body was recognizable in 19 cases: 14 Individuals were buried on either right or left side with legs bent ("Hocker") and 5 individuals were buried extended on their back. The deceased were accompanied by none or only a few grave goods: nine graves were lacking any burial goods, including the four pot burials. The other 15 graves contained pottery vessels, jewelry/personal adornments and/or animal bones and one bronze hatchet in grave 109 (*156–158*).

Individual **KEL045** (grave 97, Areal ID15, ID15:3, control-list number KL 67:411a): is a female individual of minimum 60 years at death (anthropological age and sex estimate performed by Edith Oplesch; sex estimate confirmed by aDNA-analysis). She was buried in an elongated oval, stone lined pit that was built into a WSW-ENE running wall of former building I ("Gebäude I"). The pelvis, right femur and chest area of the skeleton were strongly disturbed. The pelvis was separated from the upper body and lying 20 cm higher. The skull was fragmented and the mandible slid a bit to the side. The skeleton was oriented ENE-WSW, with the head in ENE. The deceased was buried lying on her left body side with legs flexed ("Hocker"). The left arm was extended and running diagonally from the shoulder to the knees so that the left hand was positioned between the legs. The right arm was strongly bent and the right hand resting in front of the face. A ceramic bowl was lying in front of the face in the SE corner of the grave, a ceramic juglet was placed at the right elbow. A broken

bronze pin was lying in the chest area (*157*). A tooth of this individual was radiocarbon dated to 1886-1754 cal BCE (MAMS-43549, 3498±21).

Karanaevsky ground burial, Republic of Bashkortostan, Russia (KAP002): The Karanaevsky ground burial is located 0.4 km northeast of the northern outskirts of the village of Karanaevo, on a high flat plateau on the right bank of the river Kushkain in Sterlibashevsky district (Republic of Bashkortostan, Russia). It consists of 13 mounds with diameters of 10 to 87 m, and heights of 0.2 to 1.5 m. Kurgan nb. 7 (12 m in diameter, about 0.43 m high) was excavated in 2008. It contained 6 burials. An adult man was buried in burial nb. 3, a woman in burial nb. 4, while children and adolescents were buried in others. The funeral rites and ceramics of the site are characteristic of the Srubnaya culture tribes of the Southern Urals. Burial nb. 5 contained an adolescent and ~5-year-old child (**KAP002**) buried together, together with two ceramic vessels.

"Ipogeo degli Avori", Trinitapoli, Apulia, Italy. (TRI011): The Bronze Age Madonna di Loreto site (Trinitapoli) is located in the Tavoliere delle Puglie, a plain in northern Apulia, southern Italy. The site comprises several hypogeal structures intended for cultic and, in a later phase, funerary function. The hypogea are artificial underground chambers excavated in calcareous banks and provided with a narrow and steep open-air entryway (dromos) followed by a low underground passage (stomion). At present, seven monumental hypogea are known (*159*), among which the "Bronzes Hypogeum," the "Fermatreccia Hypogeum," the "Guardian Hypogeum" and the "Ivories Hypogeum", from which comes the sample **TRI011**. The Ivories Hypogeum was excavated between 1999 and 2001 by A.M. Tunzi under the former Soprintendenza per i Beni Archeologici della Puglia. As for the other structures, the hypogeum was probably first built and used for fertility cults and ceremonies. After a phase of abandonment, during the last phase of the Middle Bronze Age, the structure underwent some structural modifications and was transformed into an elite grave (*160*).

The archaeological and bioarchaeological investigation allowed the reconstruction of the depositions' modality. In two distinct phases, dozens of corpses were progressively placed in the stomion and chamber, along with several cultural items: bronze objects, amber beads, vases, pierced shells, and two small ivory sculptures, hence the site's name (*161*, *162*). As new individuals were added, those below were displaced with a partial or total loss of anatomical connections. Thus, the skeletal series is mainly represented by commingled bones of more than 100 individuals in different preservation states. In a few cases, based on spatial proximity and biological characteristics, it was possible to assign bones to specific individuals. The preliminary anthropological analysis shows the presence of infants, children, and adults of both sexes. **TRI011** is a deciduous mandibular second molar of a 4-6 years child coming from the stomion.

Iron Age burials in Latsch/Vinschgau, South Tyrol, Italy (I0216, I0217): In 2007, several Iron Age burials were discovered during construction work in Latsch (Vinschgau). Archaeological investigations by the Amt für Bodendenkmäler, Bozen (Office for Archaeological Monuments, Bolzano) showed that, in this area, the Adige river had been dammed back several times as a result of the activity of the Tarsch debris-flow cone, creating a lake and marsh landscape. Several large stone blocks on the northern edge of the bank, broken off from the steeply rising rock face, formed the reference point for inhumation burials of the Iron Age. The bone material recovered during archaeological investigations indicates a minimum number of 14 individuals, including 10 adults and 4 children. The burials contained remarkably little jewelry and grave goods: a single amber bead, finger

rings, small rings worn in plaits, a bronze bracelet, part of a ceramic vessel and animal bones (sheep/goat, pig, cattle, dog). In addition to typological dating of the findings, which placed the burials in the Hallstatt and Early La Tène periods, a series of 14C dating was carried out: 800 - 480 cal BCE (91.1%); 780 - 480 cal BCE (87.3%); 770 - 410 cal BCE (95%); 600 - 390 cal BCE (71.3%); 400 - 160 cal BCE (95.4%).

Burial practices appeared to depend on the available space. Stone linings or covers were not observed. One woman was probably buried in a crouched position (Hocker) in a niche, while another individual was buried laterally in a crouched position (Hocker) with arms bent. At least two individuals, an adult woman and a child were buried together with their legs intertwined. Some of the skeletons were completely surrounded by river sediments, indicating that the river had risen at least temporarily above the burial level, altering the original body positions. It is remarkable that the deceased were not buried according to the local cremation practices of the Fritzens-Sanzeno culture typical for South Tyrol, North Tyrol and Trentino, which suggests that these individuals were part of a group of settlers who had migrated to the Vinschgau Valley and retained the burial customs of their place of origin.

Chotín, Komárno district, Slovakia (CHT001): At Chotín, two Celtic cemeteries were found. The first La Tène (LT) cemetery was found southeast of the village (site VIII) during rescue excavations led in 1960-61 by M. Dušek (IA SAS). 16 burials were initially discovered, but more graves were revealed and destroyed east of the excavated area as a result of sand extraction conducted in 1965, three of which were inventoried by Priska Ratimorská. In 1975, investigations led by J. Bujna led to the discovery of four additional graves, one of which contained burial goods. The graves date from the end of the LT B1 to the LT B2 periods, *i.e.* to the last quarter of the 4th to the first half of the 3rd century BCE.

Another biritual LT cemetery was found west of the village, at "Chotín X". Initial graves were discovered and destroyed during sand extraction, but the inventory could be saved for three of them (nb. 1-3). During rescue excavations led in 1971-72 (Podunajské Múzeum Komárno, under the direction of P. Ratimorská), 44 additional graves were excavated (*163*). Among the total of 47 graves discovered, only four are cremation graves. Five graves were weapon-equipped tombs, and an iron sword in a scabbard was found in all of them. In a warrior's grave (nb. 14), a two-piece bronze arm bracelet with a hinged closure and rich filigree decoration was found. The grave of a rich woman (nb. 21) several pottery items were found, including a vessel with a profiled foot of Hellenistic style (a kantharos). In grave nb. 34, a man was buried with a gold and a silver ring on the left hand as well a set of tools comprising an axe and three files (*164*). Further analyses of the skeletons and as well as genetic investigation have been conducted on this site (*164–168*). Individual CHT001 was a 23 to 32-year-old male which was buried in grave 12 (excavated in 1971) at the site Chotín "X". It was found with two bronze and two iron made fibulae close to the upper part of the body, a bronze (?) bracelet at the right upper arm, two iron "Koppelringe" around the pelvic region, two bronze hollow casted buckle anklets at the feet as well as a ceramic vessel.

Akbeit burial ground, Kazakhstan (AKB003): The large Akbeit burial ground belongs to the Tasmola culture, which developed in the steppes of Central Kazakhstan during the 8th-5th centuries BCE (*169*). Studies have highlighted connections between the Tasmola population and tribes of eastern regions of Central Asia (*170*), showing that early Saka cultures were much closer to each other than previously thought. Individual **AKB003** was found in a grave in Kurgan 7, which is located in the first of the four groups of the Akbeit burial ground, and has a diameter of 21 m and a height of 2.5 m. The grave had the following dimensions: length 1.9 m, width 1.8 m, and depth 0.95 m. The skeleton, belonging to a 4 to 5-year-old child, was found at the bottom of the grave. Objects found with the skeleton such as

a gold necklace, two gold earrings with turquoise inserts, a bronze mirror decorated with gold, and a small bone case likely containing a red cosmetic substance, suggested that the individual was a female belonging to the elite of the Tasmola society (*171*, *172*).

Berel necropolis, Kazakhstan (BRE008,BRE026 and BRE028): The Berel necropolis was discovered around elite kurgans of the Pazyryk time, and has later revealed materials identified as belonging to the Hun-Xianbei cultural-chronological horizon (1st-4th century CE), which fits into the broad framework of the so-called Great Migration of Peoples era (2nd century BCE - 6th century CE) (*173*). To date, it encompasses over 50 mounds of different sizes, half of which are either cenotaphs or altar, and the others containing simple shallow burials in soil pits, stone boxes or wooden structures. These various burial structures, together with different orientations of the deaths, and the occasional inclusion of horses in the burials, point to ethnocultural diversity. In particular, some of the materials from the Hun-Xianbei period found in the site exhibit direct analogies with the Transbaikal region.

Individual **BRE008** (Berel 2017_90A) was excavated from a ~3 m diameter mound, surmounted by an irregular circle of stones. The skeleton was found at a depth of 60 cm, in an elongated position on the back, with the head oriented to the west. The right clavicle and bones of the right hand appeared to have been moved, most likely by rodents. The left foot was also missing. The only objects found with the skeleton were sheep bones and a corroded iron object (probably a meat knife). The individual was identified as a female and radiocarbon-dated to 258-366 cal AD (MAMS-42126 1730 ±13). Individual **BRE026** (Berel 13) was found in burial nb. 13, in which the remains of two horses were also discovered. The burial also contained a set of weapons characteristic of a lightly armed equestrian warrior from the Hun-Xianbei period of the Early Medieval Kazakh Altai (*173*). Individual **BRE028** (Berel 2) was excavated from an elite burial mound from the Pazyryk period, of ~28 m diameter and 0.7 m height (mound nb. 2). It contained a large chamber (4.9x4.0 m, more than 4 m depth), which itself contained a wooden funerary cabin in which the remains of the individual were found. Several objects were also found in the burial, including clothes decorated with gold foil, a bronze mirror with a zoomorphic handle, a grater made of light gray fine-grained granite, grains of cereals, ceramic vessels, a wooden table, a horse tail and an iron knife. The funerary chamber also contained the remains of seven horses found with golden ornaments depicting fantastic polymorphic creatures near the head. These elements are characteristic of the 4th-3rd c. BCE in Berel.

Kuelap archaeological complex, Chachapoyas, Amazonas, Peru (KUE033): The Kuelap archaeological complex belongs to the Chachapoya culture, and is located in the Tingo district, Luya province, Amazonas region, at 3000 m asl. The Chachapoya culture flourished around 800 CE until conquered by the Inca and the Spanish. Kuelap, located at 3000 m asl on the left bank of the Utcubamba river, is one of the largest sites of this kind (~450 ha), which is composed of public and residential buildings and also includes funerary areas and agricultural terraces as well as massive perimeter walls. The perimeter walls enclose various differentiated areas with about 420 structures, most of which exhibit a circular layout. The functionality of the site is still debated. Due to several architectural features, its location and surroundings, it has been suggested as a fortified city, a residence of a centralized elite and even a center of astronomical importance (*174*). There have been various seasons of archaeological work in different areas of the site since the 1980's and until now, which have recovered hundreds of skeletonized human remains. **KUE033** comes from the "La Fortaleza" area (South Sector, Structure 3, E3, burial 12A) and was excavated in 1989. The preliminary bioanthropological assessment made during sample collection suggested it is an adult male.

Purunllacta de Soloco, Chachapoyas, Amazonas, Peru (PLS004): The site is located in the Sonche valley at an altitude of around 3000 m asl. Purunllacta de Soloco and other sites in this region show cultural features (pottery and architecture) that indicate Chachapoya or Late Intermediate (1000-1475 CE) and Late Horizon or Inca (1475-1532 CE) occupations; however, the site also has evidence of occupation during the colonial period following the Spanish conquest. This site and others situated on the right bank of the Utcubamba river were integrated to the Qhapaq Ñan Inca road system to a certain degree. In addition to buildings with a circular layout, typical of Chachapoya settlements, there are structures in "D" shape, which have also been found elsewhere in the region, e.g. Kuelap. This architectural style seems to have been frequent during the Late Horizon and their function as ceremonial structures has been suggested. The site has two sectors, the first (Sector A) at a lower elevation and associated with the Inca road, and the second (Sector B) at a higher elevation (ridgetop) (*175*). The site was excavated in 2014 and 2015 and researchers recovered artifacts and human remains associated with the Inca and colonial periods. **PLS004** was recovered from Sector A, unit 14 (Nivel 3, Rasgo 4, burial 7). The preliminary bioanthropological assessment made during sample collection suggested it is an adult female.

The Malaya Ryazan II settlement, Samara region, Russia (MLR005): This site is located on the right bank of the Volga river in the southern part of Samarskaya Luka (Samara region). It extends over about 30 hectares. The cultural layer of the Middle Ages dates to the 13th-14th century CE. The settlement was a trade and craft village of the Ulus Jochi (Golden Horde), in which a predominantly Russian population lived (*176*). A cemetery was located in the eastern part of the village. In 2007-2012, 131 burials were excavated. All adult burials were males, with the exception of 2 females, and 63 burials were children. **MLR005** (Burial 15) was a ca. 55-year-old male, laid on his back. The lower jaw was displaced, and some bones were partially destroyed.

Mayachny Bugor ground burial, Astrakhan region, Russia (MAY017): The ground burial "Mayachny Bugor" is located 400 meters north of Krasny Yar village outskirts (Astrakhan region, Russia), on the eponymous Baer knoll, and is conventionally divided into 4 parts: Mayachny Bugor-I, II, III and IV. **MAY017** was found in burial 80, which belongs to Mayachny Bugor-II, on the northeastern part of the knoll, and is a burial pit with an irregular trapezoidal shape and a rounded eastern wall. (~ 116x30 cm). Wooden remains were found in the pit. The individual was a 5.5-6-year-old child, laid on his back, with the head directed to the west-northwest. The skull was crushed by the soil, but it could be determined that it was laid face up and slightly inclined to the right. The left half of the skeleton was destroyed, likely by animals, with the bones shifted from their original position. The right arm was extended closely along the body, with the wrist close to the right-wing of the pelvis. The legs were stretched out. The bones showed signs of iron deficiency anaemia. This burial belongs to the Golden Horde period (13th-14th century CE).

Ancient HBV genome reconstruction

The number of reads screened per library prior to HBV DNA enrichment ranged from 1.3M to 1,485M (median=5.1M). The fraction of HBV-mapping reads in positive individuals ranged from 0.01 to 77.95 reads/M (median=0.78/M). After targeted DNA enrichment, the fraction of HBV-mapping reads increased by several orders of magnitude (median: 47,377-fold), which allowed us to reconstruct a significant fraction of the HBV genome in most cases. On average, 72% of the genome was covered at least 3x, and that proportion exceeded

90% in 66 individuals from which almost complete genomes could be reconstructed (fig. S9A,B, Data S1).

Typical patterns of ancient DNA damage were observed in most cases, with an average C to T substitution rate at the 5' read end of 19.3% and 6.4% for non-UDG and half-UDG treated libraries, respectively (fig. S9E; Data S3). The frequency of damage-like substitution observed on HBV-mapping reads correlated well with that observed on human-mapping reads across libraries (R-squared: 0.71; fig. S9F). When only a low number of HBV-mapping reads were recovered, or when a full UDG treatment was employed for library preparation, it was not possible to properly estimate DNA damage. However, sample contamination with HBV DNA is unexpected, and the phylogenetic placement of all reconstructed HBV genomes was consistent with their spatio-temporal origins, supporting their authenticity (fig. S3; table S2).

Teeth are generally considered as the best DNA reservoir for ancient bloodborne pathogens (*177*, *178*). DNA libraries prepared from teeth indeed yielded significantly higher HBV genome coverage than those prepared from bones (ratio of estimated marginal means: 1.9, p-value: 0.0071; fig. S9C). However, it is remarkable that complete HBV genomes could be recovered from a variety of skeletal elements, including from the low vascularized petrous bone. Additionally, no significant difference of coverage was detected between libraries prepared from petrous and other bones (p-value: 0.99).

The HBV genome is partially double-stranded, with a single-stranded portion of variable length covering about one fourth of the genome (*69*). On average, coverage was ~6 times higher in the double stranded portion of the genome compared to the single-stranded region (fig. S9B). However, a significant proportion of the single-stranded region could be reconstructed in most cases, even when a double-stranded protocol was used for library preparation. This is consistent with variation in length of the single-stranded region among circulating viral particles, some of which can exhibit completely double-stranded genomes (*69*). Using a single-stranded instead of a double-stranded protocol for library preparation led to an increase of HBV genome coverage (ratio of estimated marginal means: 6.2, p-value: 3e-4; fig. S9D). On the other hand, the use of a single-stranded library protocol did not lead to a significant reduction of the coverage ratio between double and single-stranded regions (p-value: 0.061). This might, however, be due to a lack of detection power, since only 8 out of 154 libraries were prepared using a single-stranded protocol in our study.

Phylogenetic analyses

Model selection strongly supported the use of a relaxed clock instead of a strict clock model, and the use of a coalescent skyline population model with 5 time intervals was favored (table S5). Bayesian estimation of temporal signal showed overwhelming support for the model incorporating tip dates (log Bayes factor: 338), indicating a strong molecular clock signal in our dataset, consistent with the results of root-to-tip regression (fig. S3). We estimate an HBV substitution rate of 1.5e-05 subst.site-1.year-1 (95%HPD: 1.3e-05-1.7e-05) and a tMRCA of 13,518 BP (95%HPD: 12119-15680). These estimates were relatively robust with respect to different aspects of the dataset (table S1). On the other hand, the use of a time-dependent rate (TDR) model yielded a significantly higher tMRCA (95% HPD: 17170-20240).

Our estimated maximum clade credibility (MCC) tree exhibits the most frequently reported topology so far, in which the oldest split separates the American genotypes from those recovered in the rest of the world (*18*) (which we refer to as the Eurasian branch). The monophyly of the American HBV branch, including all ancient American strains dating back to as far as ~9 ka, was highly supported (Fig. 2). Ancient strains from western Eurasia were either grouped with modern genotypes A or D, or formed distinct clades that appeared

consistent with their spatiotemporal distribution: Mesolithic clades 1 and 2, Anatolian Early Neolithic branch (composed of a single genome) and the Western Eurasian Neolithic-to-Bronze Age clade (WENBA, from which genotype G appears to descend). The respective monophyly of all these lineages, as well as that of the other modern genotypes, was highly supported (Fig. 2). Conversely, deep branching patterns were not well resolved. In particular, the monophyly of the Eurasian HBV branch was not well supported, and the exploration of plausible alternative topologies pointed to the possibility that some of the Eurasian lineages had diverged before the American branch (fig. S4). The placement of modern Eurasian genotypes relative to ancient lineages also appeared uncertain: while genotypes A, B, C, D, E and I formed a clade branching off between the Mesolithic 1 and later ancient lineages when using the full dataset, they appeared as a sister group to all ancient lineages when excluding modern data or genomes recovered from individuals showing signs of mixed infections, and the placement of this clade was not well supported in any analysis (fig. S5). Furthermore, the clade formed by genotypes D and E was recovered within the WENBA clade after exclusion of identified recombining regions (fig. S5D), which suggests that the polymerase region of these genotypes descends from a WENBA strain. Finally, the placement of genotype A relative to genotypes B and C also appeared poorly resolved.

Although these phylogenetic ambiguities do not question the main conclusions of this study, they highlight that further work is needed to decipher the early phylogeographic history of HBV and to decipher the evolutionary origin of the main genotypes prevailing today. Of note, the use of a TDR model yielded 100% support for the monophyly of the Eurasian branch, and a dating for the split of the Eurasian and American branch that was more in line with current estimates for the genetic divergence of ancestral First Americans (fig. S5B). Therefore, while model selection favored the use of a relaxed clock model (log BF: 405), the TDR model appeared to yield more consistent deep branching patterns. Furthermore, the TDR was selected over a strict clock model (log BF: 29). Thus, models accounting for both time-dependency and inter-lineage variation of rates would certainly be useful for further investigation. Finally, the use of phylogenetic methods explicitly accounting for genetic recombination, as well as the reconstruction of additional ancient HBV genomes from more eastern parts of Eurasia may also provide better resolution regarding these questions in the future.

Genotyping of ancient HBV strains

We used the *mPTP* species delimitation tool (*179*) to classify ancient HBV strains into genotypes, as previously described (*180*). The method grouped all modern genomes in accordance with conventionally defined genotypes, with two exceptions: (i) genotype C and I were grouped together, consistent with previous observations that the genetic distance between some strains of these genotypes is below the usual delimitation threshold (*3*), (ii) genotype J was grouped with lineages found in gibbons and orangutans.

All ancient strains recovered on the branch leading to genotype A and D were classified as such, except for RISE387, which was assigned its own genotype. The clade formed by KVK001 and younger Mesolithic genomes from Europe as well as the one recovered from an early farmer of Anatolia (BON020) were classified into two respective genotypes, while the two oldest Mesolithic genomes (MPR001 and MN2003) were grouped with Southeast Asian NHP lineages and genotype J. The WENBA clade was classified into ten different genotypes, with genotype G identified as a separate genotype. The two most ancient American strains (CUN002 and CAO009) were assigned their own respective genotypes, while two strains from Peru dating back to ~500 years ago. were grouped together. Two other strains from the same period found in Peru and Mexico were grouped with genotypes F and H respectively.

The examination of interstrain genetic distances highlighted difficulties in reconciling conventionally used genotype delimitation thresholds (>7.5% intergroup divergence across the complete genome (*3*)) with a time-continuous phylogenetic framework (table S4). Indeed, ancient genomes may represent intermediate evolutionary steps, blurring frontiers between genetic groups that appear consistent when looking at modern diversity alone. While ancient strains classified as genotype A or D by *mPTP* indeed showed less than 7.5% divergence with any modern strain of the respective genotype (except for RISE387), they also showed <7.5% divergence with ancient and modern strains from other genotypes. In general, grouping ancient strains based on the standard approach would have resulted in the delimitation of genotypes that did not form monophyletic lineages. For instance, the genetic distance between any European Mesolithic or Anatolian Early Neolithic strains didn't exceed 5.3%, but those formed three separate lineages. While the WENBA clade (excluding genotype G) was classified into 9 genotypes by *mPTP*, the maximum interstrain divergence in that entire clade was 8.6%.

While phylogeny-aware tools such as *mPTP* can represent interesting classification alternatives in this context, they are sensitive to phylogenetic uncertainties. For example, some of the WENBA subclades classified as genotypes were poorly supported, and not recovered as monophyletic in our time-calibrated phylogeny. Therefore, we used a provisional classification scheme for ancient strains based on phylogenetic clades that appeared robust to phylogenetic uncertainty and reflected a consistent spatiotemporal distribution: Mesolithic lineages 1 and 2, Anatolian Early Neolithic lineage, Western-Eurasian Neolithic-to-Bronze-Age (WENBA) lineage, Ancient American lineages 1,2 and 3 (fig. S2). Ancient strains grouping monophyletically with a specific modern genotype were classified as such. Using *EPA-ng*, we were able to assign modern genotypes or previously defined ancient HBV lineages to all low-coverage genomes except one (table S2; fig. S3). Likelihood weight ratios were close to 1 in the vast majority of cases, and all assigned lineages appeared consistent with the spatio-temporal context of corresponding individuals. The only genome which couldn't be assigned to a lineage was recovered from an individual from Tajikistan dated to ~8.2 ka, which appeared to belong to an ancient lineage that is not represented in our phylogeny (fig. S3).

Recombination analysis

Homologous genetic recombination is known to occur in HBV, and it is largely accepted that entire HBV genotypes descend from genomic hybrids (*181*). An exploratory search performed using *RDP4* (*84*) revealed signals for 30 putative recombination events in our dataset. Further investigation of the results allowed to retain 5 well supported events (fig. S10). These included previously reported findings (*181–183*): (i) we identified genotype E as descending from a recombinant between an unknown lineage and genotype D, (ii) we identified the clade formed by subgenotypes B2, B3 and B4 as descending from a recombinant between subgenotype B1 and genotype C, (iii) we identified genotype I as descending from a recombinant between genotype C and an unknown lineage, and (iv), we identified a genome isolated from a wild chimpanzee in East Africa (AN: AF498266) as a recombinant between a typical chimpanzee strain and human genotype C. For all these recombination events, except the last one, identified recombination breakpoints were around the edge of the polymerase gene.

Previous analyses of ancient HBV genomes suggested that genotype A originated from a recombination event between an unknown lineage and genotype D around the polymerase gene (*14*). We recovered a well-supported signal for a recombination event involving genotypes A and D in the same genomic region, which was recovered in all ancient and modern genomes belonging to these respective genotypes. However, although genotype A

was marked as the recombinant in the initial exploratory search, possible misidentification of recombinant and parental genomes was indicated. Examination of local ML trees rather pointed to genotype D being the recombinant, since it appeared as a monophyletic clade within genotype A in the tree corresponding to the recombining portion of the genome. Additionally, a phylogenetic analysis excluding the identified recombining region in genotype D suggests that its second parental strain belonged to the WENBA clade (fig. S5d). However, these results must be taken with caution, since the reconstruction of local phylogenetic trees could suffer from biases related to other recombination events, as well as from the loss of overall phylogenetic signal.

Our results are in accordance with the established view that the genetic diversity of HBV was largely shaped by genetic recombination, and that several of the main HBV genotypes prevailing today descend from ancestral recombinants. We employed a conservative approach to identify a set of well supported recombination events. However, the latter cannot be regarded as exhaustive. In particular, the initial exploratory search suggested several putative recombination events involving genomes from the WENBA clade. However, none of these was supported by all methods or could be independently reconciled with a consistent evolutionary scenario based on local phylogenetic topologies. This highlights the difficulty to disentangle the evolutionary history of this virus which likely encompasses multiple and successive recombination events affecting overlapping regions of the genome. Ancient HBV genomes contain precious information in this regard, since they may belong to lineages involved in past recombination events that are more difficult to detect after thousands of years of evolution. Nevertheless, recombination analyses such as the one conducted here are unable to provide comprehensive phylogenetic pictures. Conversely, although classical phylogenetic methods are useful for describing and dating the main evolutionary divergences of this virus, they cannot capture the full reticulated process. In this regard, the use of phylogenetic models explicitly accounting for genetic recombination should be helpful in the future.

Genomic characteristics of ancient HBV strains

Some of the ancient strains from the WENBA clade carried an insertion close to the 5' end of the C gene associated with a stop codon in the pre-core region, similarly to genotype G. This was exclusively observed in 14 strains forming the subclade from which genotype G descends in our phylogeny. The insertion carried by most of these ancient strains was 39-nt-long and appeared non-homologous to the insertion found in genotype G in part of its sequence (fig. S8). However, in the two Bronze Age genomes which appeared the most closely related to genotype G (SGR003 and VLI060), the insertion was 36-nt-long and strictly identical to the one found in genotype G.

The 6-nt insertion typically found in the core gene of modern genotype A strains was recovered in several ancient strains belonging to genotype A, dating back to as far as ~2 ka. (SJN001, Abusi1543, DA119 and I1321), but not in earlier strains from that lineage. This points to the acquisition of that insertion shortly before the divergence time of all modern genotype A subtypes, which we date to around 3 ka.

The length of the pre-S1 region of the HBV genome varies across genotypes due to the presence of large indels near the 5' end of this reading frame (*3*). The shortest pre-S1 region is found in genotype D as well as non-human primate genotypes, and has a typical length of 324 nucleotides. This was also the case for all the HBV genomes dating back to between ~11 and ~7.5 ka. (i.e. the oldest recovered in this study), as well as many genomes in the WENBA clade (fig. S8). This suggests that the MRCA of all HBV lineages carried this form of the pre-S1 region, and that insertions must have occurred later on, contrasting with the classical perspective in which genotype D and NHP strains exhibit a 33-nt deletion in this

region (*3*). Instead, it appears more phylogenetically consistent to say that genotypes A, B, C and I carry a 33-nt insertion (also found in all ancient relatives of genotype A) which must have existed in their most recent common ancestor. Genotype F and H exhibit another 33-nt insertion at the same genome location, which we recovered in all ancient American genomes except the oldest one (CUN002). Other types of pre-S1 insertions, located near the 5' end of the reading frame, were found among WENBA HBV strains: a 12-nt insertion found in 33 strains, a 30-nt insertion found in four of them (MK5004, MK5009, LEU065 and PDA003), and a 30-nt insertion found in genotype G and its closest ancient relatives (SGR003 and VLI060). Additionally, 42 genomes from the WENBA clade contained a 36-nt insertion around position 224 of the pre-S1 region (using genotype D numbering), and the oldest American strain (CUN002) carried a 12-nt insertion at the same position.

Most of the above-mentioned insertions were observed in sets of genomes forming monophyletic groups in the phylogeny, further supporting the reconstructed topology (of note, all regions containing insertions were masked from the alignment used for phylogenetic analyses). However, two of them were found to be shared between distant lineages, suggesting ancestral recombination events that were not detected during the recombination analysis: three genomes from the WENBA clade (KAP002, MIB011 and RISE154) carried the 33-nt insertion found genotypes A,B,C and I, and the 30-nt insertion found in genotype G was also found in genotype E.

Origin of HBV in non-human apes

Non-human hominoids have also been found infected by HBV, with two specific HBV lineages infecting African (chimpanzees and gorillas) and Southeast Asian apes (gibbons and orangutans) respectively. Most HBV phylogenies recover these non-human-primate (NHP) HBV lineages at interspersed positions between human-specific genotypes, which has been thought to reflect separate spillover events from humans to wild primates in the past (*7*, *28*). Our phylogenetic reconstruction and dating also supports this idea since NHP lineages are recovered as two separate clades branching within the Eurasian branch (Fig. 2). An alternative scenario involving two HBV lineages coevolving with NHPs and being transmitted independently to humans seems incompatible with the timescale of the HBV phylogeny, which we estimate to be ~3 orders of magnitude shorter than that of hominoids (*184*). Other intermediate hypotheses involving several NHP-to-human and human-to-NHP transmission events are possible but appear less parsimonious given currently available data.

Southeast Asian and African NHP lineages are recovered as a sister clade to the Mesolithic clades 1 and 2 (although with low support in the first case), with median estimates of divergence times around ~11 and ~12 ka, respectively. This may indicate that HBV was transmitted to NHP by hunter gatherers around that period. HBV transmission from humans to wild NHP might have involved contact with infected body fluids during aggressive interactions (*185*, *186*), which could indeed be more likely to occur in human populations relying largely on hunting for subsistence (*187*, *188*). However, these divergence times represent earliest estimates for the date of these potential human-to-NHP spillover events (*189*). Physical interactions between humans and NHPs in these regions have likely continuously occurred and still happen today (*190–192*). Additionally, given that the distribution of NHPs has likely not changed dramatically during the past ten thousand years, these putative spillover events should have happened in African and Southeast Asian rainforests respectively. Since NHP HBV lineages branch off from lineages recovered in Europe in our phylogenetic reconstruction, a significant part of the biogeographic history leading to the spread of the virus into these Asian and African apes remains to be revealed. Furthermore, it has been shown that accounting for host-specific differences in viral evolutionary rates may be important for robust phylogenetic reconstruction (*193*). Thus, in

the future, the retrieval of HBV genomes from ancient NHP specimens combined with the use of host-specific clock models could be helpful to further assess and understand the phylogenetic placement of NHP HBV lineages.

Origins and spread of genotypes A and D

The oldest ancient strains belonging to genotype A in our dataset are found in individuals from western Russia and the northern Caucasus dated between ~5 and ~4 ka (SGR004, RISE386/387, KBD002, KDC001; fig. S1). Around 2.5 ka, genotype A is observed in two Scythian individuals from the Pontic steppe (I0960) and the Carpathian basin (DA195), a region where it is still found later on in individuals dated to ~2.1 and ~1.5 ka, respectively (CHT001, DA119). As stated previously (14), these findings fit well with the known genetic relationship between western Scythians and Bronze Age populations of the western steppe (194), and suggest that the presence of this genotype in Africa today is due to later dispersal from Eurasia. The latter point is further supported by the phylogenetic position of a strain discovered in an Egyptian mummy dated around 1.9 ka (15) (Abusir1543), which appears basal to subgenotypes typically found in Africa today (A1 and A3; fig. S2). However, our results also reveal the presence of this genotype in northeastern Europe (BOO006/008) as well as in the Near East (ALA098/110, KEL045) between ~4 and ~3.5 ka, bringing additional mystery regarding its precise geographic origins and early dissemination routes.

Ancient HBV strains belonging to genotype D appear during the Iron Age in our dataset, with first occurrences dated around 2.5 ka in two individuals from the same site in today's Italian Alps (I0216 and I0217; fig. S1), as well as from different parts of the western steppe ranging from southwestern Russia to Central Asia (ORE002, AKB003, BRE026/028, DA51). From the early Medieval period on, this genotype is observed in large parts of Europe where it appears to have become predominant (Fig. 3), which it still is today (4). Although the geographical distribution of genotype D prior to the Iron Age cannot be directly assessed using our dataset, the apparent absence of this genotype in western Eurasia before that period, together with its wide presence in Central Asia, suggest an Asian origin followed by east-to-west expansions (Figs. 3, S1). However, recombination analyses indicate that this genotype might descend from a genotype A/WENBA hybrid, although this result should be viewed with caution (see "Recombination analysis" section; figs. S10, S5d).

Ancient genotype D strains from Europe do not form a monophyletic group in our phylogenetic reconstruction, which could suggest several waves of dissemination (fig. S2). Iron age genomes from Central Asia appear basal to most European Medieval genomes, which may reflect westward migrations across Eurasia occurring during the Migration Period (195). In contrast, earliest genotype D strains from the Italian Alps form the deepest genotype D branch, pointing to a distinct origin. Finally, several genotype D strains from Medieval Europe are found basal to subgenotypes which also prevail in other continents today (D3, and D4), suggesting that European colonization and modern migrations might have driven the spread of these lineages.

Fig. S1.
Geographic locations, date range (ka BP) and lineage of ancient Eurasian HBV genomes. Previously published genomes are labelled in light grey.

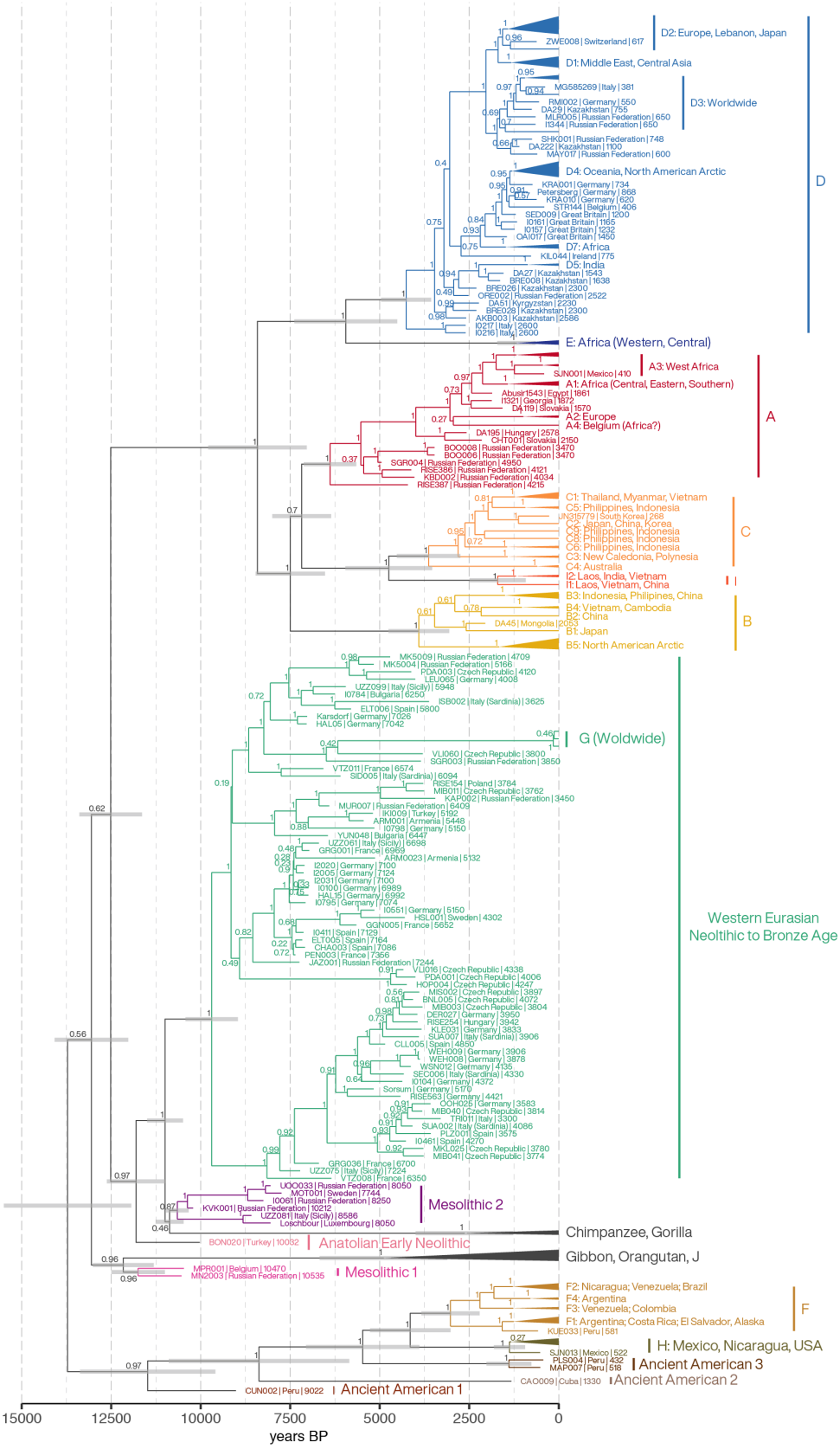

Fig. S2.
Time-calibrated phylogenetic tree of HBV. Sample labels are displayed for ancient genomes, together with their geographic locations and midrange date estimates. The main geographic

distribution of each (sub)genotype is indicated. Posterior node support and age estimates (grey bars indicating 95% highest posterior density; except within genotypes) are reported.

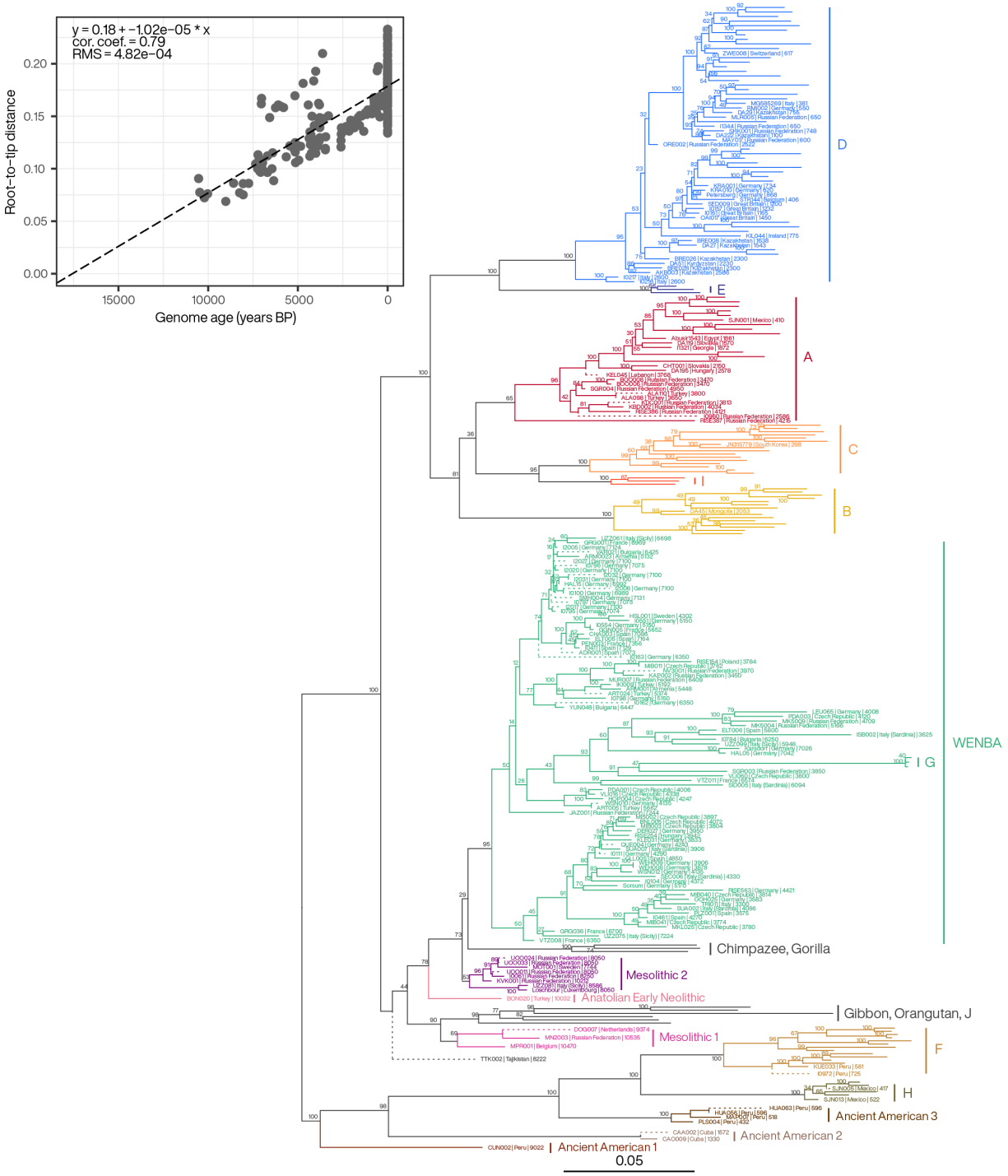

Fig. S3.
Phylogenetic tree with branches in substitutions per site estimated by maximum likelihood. The tree was midpoint-rooted. Bootstrap supports are reported on the nodes. The most likely phylogenetic position of low-covered genomes, as estimated with EPA-ng, are represented by dotted lines (note that these were not used for the construction of the tree). In the upper left panel: regression of root-to-tip genetic distances against tip dates.

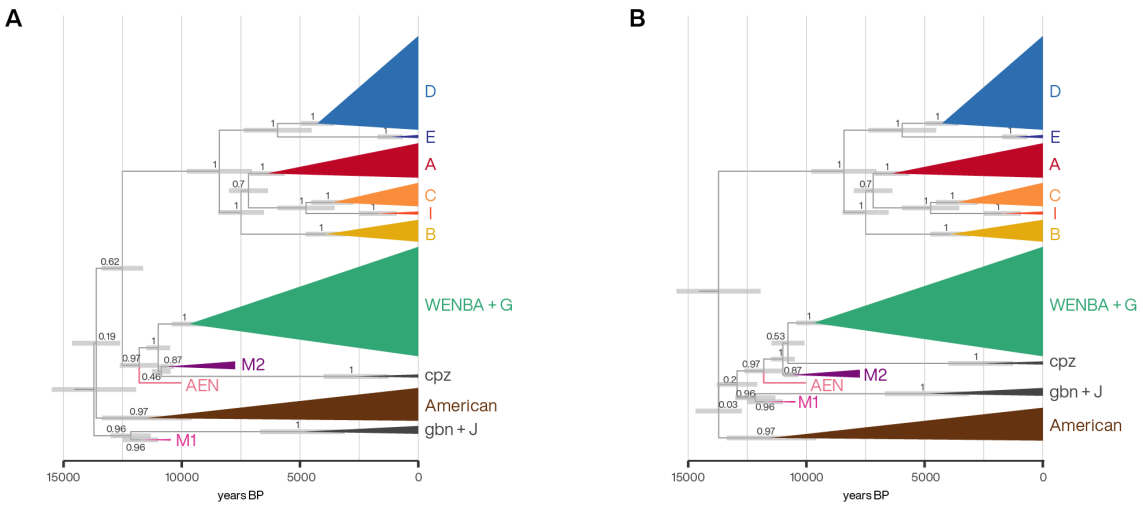

Fig. S4.
Time-calibrated phylogenetic trees of HBV representing alternative topologies for two low-supported clades: (**A**) the Eurasian branch and (**B**) the clade corresponding to the Eurasian branch with the exclusion of the Mesolithic 1 and Southeast Asian NHP clades. MCC trees conditioned on the absence of these clades were retrieved, and the full posterior tree sample was used to compute node posterior supports.

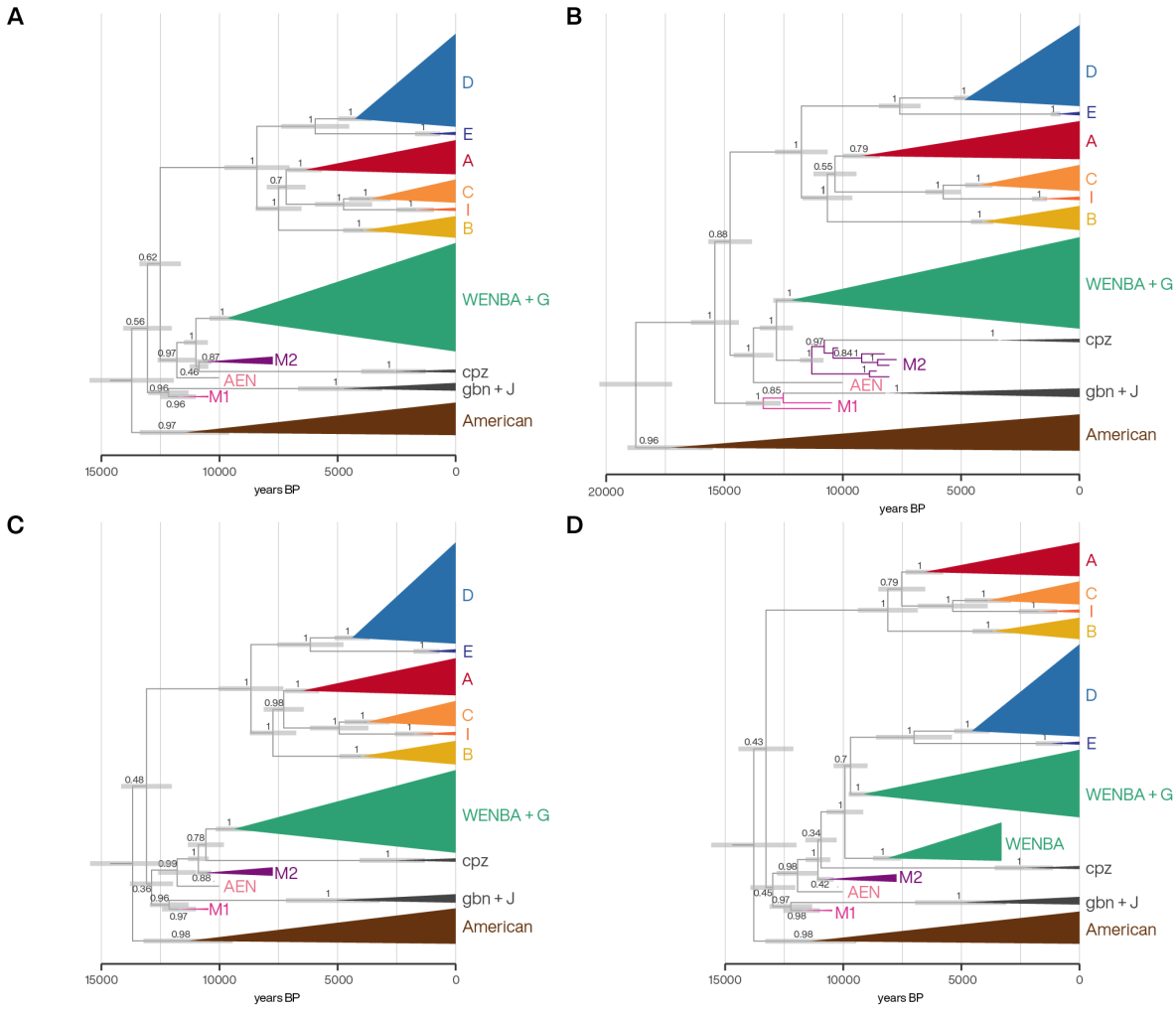

Fig. S5.
Time-calibrated phylogenetic trees of HBV reconstructed using different subsets of the data: (**A**) full dataset, (**B**) exclusion of modern genomes, (**C**) exclusion of genomes recovered from mixed infection contexts, (**D**) masking of recombining regions identified with RDP4. All analyses were performed using a skyline coalescent tree prior with five time intervals, and a lognormal relaxed clock.

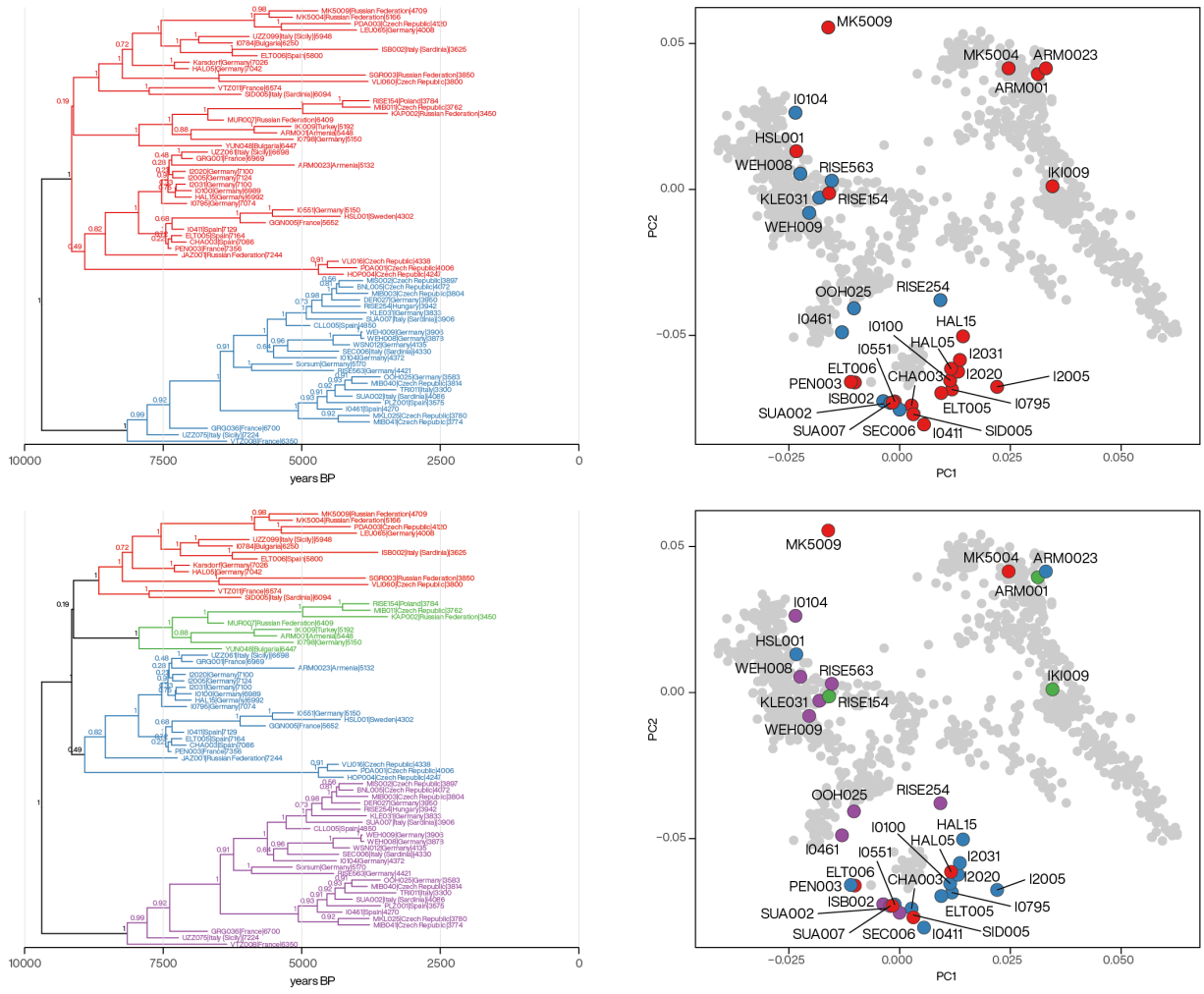

Fig. S6.
Exploration of potential correlations between the phylogenetic relationships of ancient HBV genomes within the WENBA clade and the genetic profile of the individuals carrying them. On the left: a subset of the time-calibrated phylogenetic tree corresponding to the WENBA clade (genotype G excluded) is colored according to two sublineage classification schemes. On the right, the principal components analysis plot of modern and ancient western Eurasians summarizes the genetic variation of a subset of individuals for which human genetic data was available. Individuals are colored according to the lineage of the HBV strain they carried, as in the tree on the left.

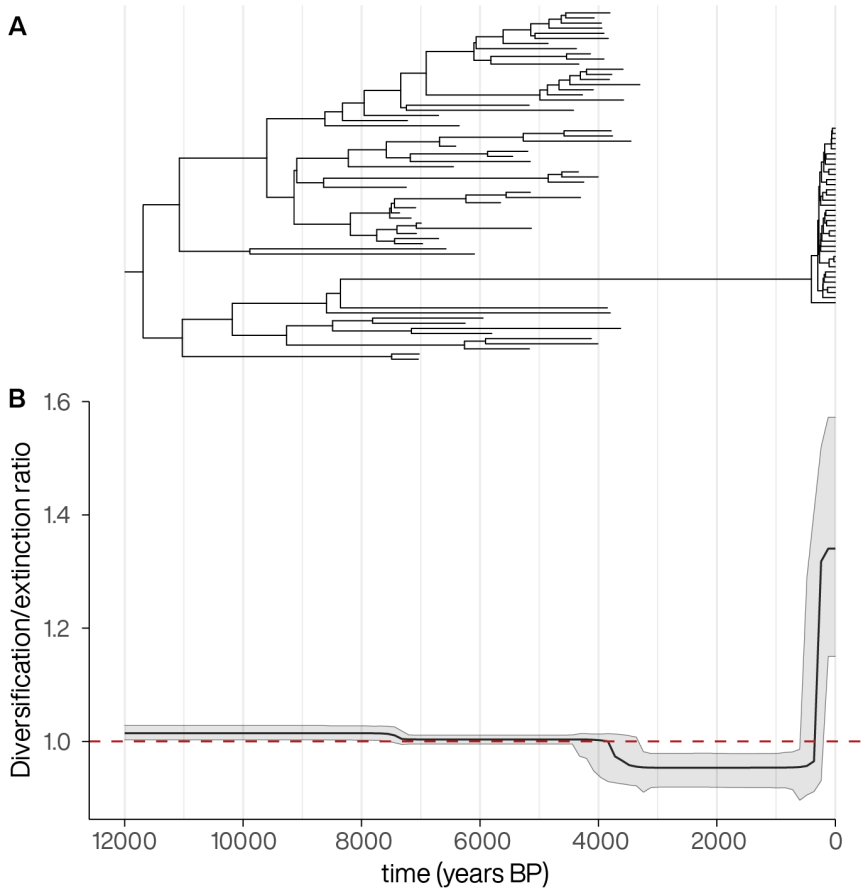

Fig. S7.
Phylodynamic analysis of the WENBA lineage. (**A**) Time-calibrated phylogenetic tree obtained using a birth death skyline tree prior and a strict clock model, with a dataset consisting of ancient WENBA strains and modern genotype G strains. (**B**) Skyline plot showing the estimated diversification/extinction ratio d through time with 95% HPD. The red dotted line indicates d=1, below and above which the number of infections decreases or increases, respectively.

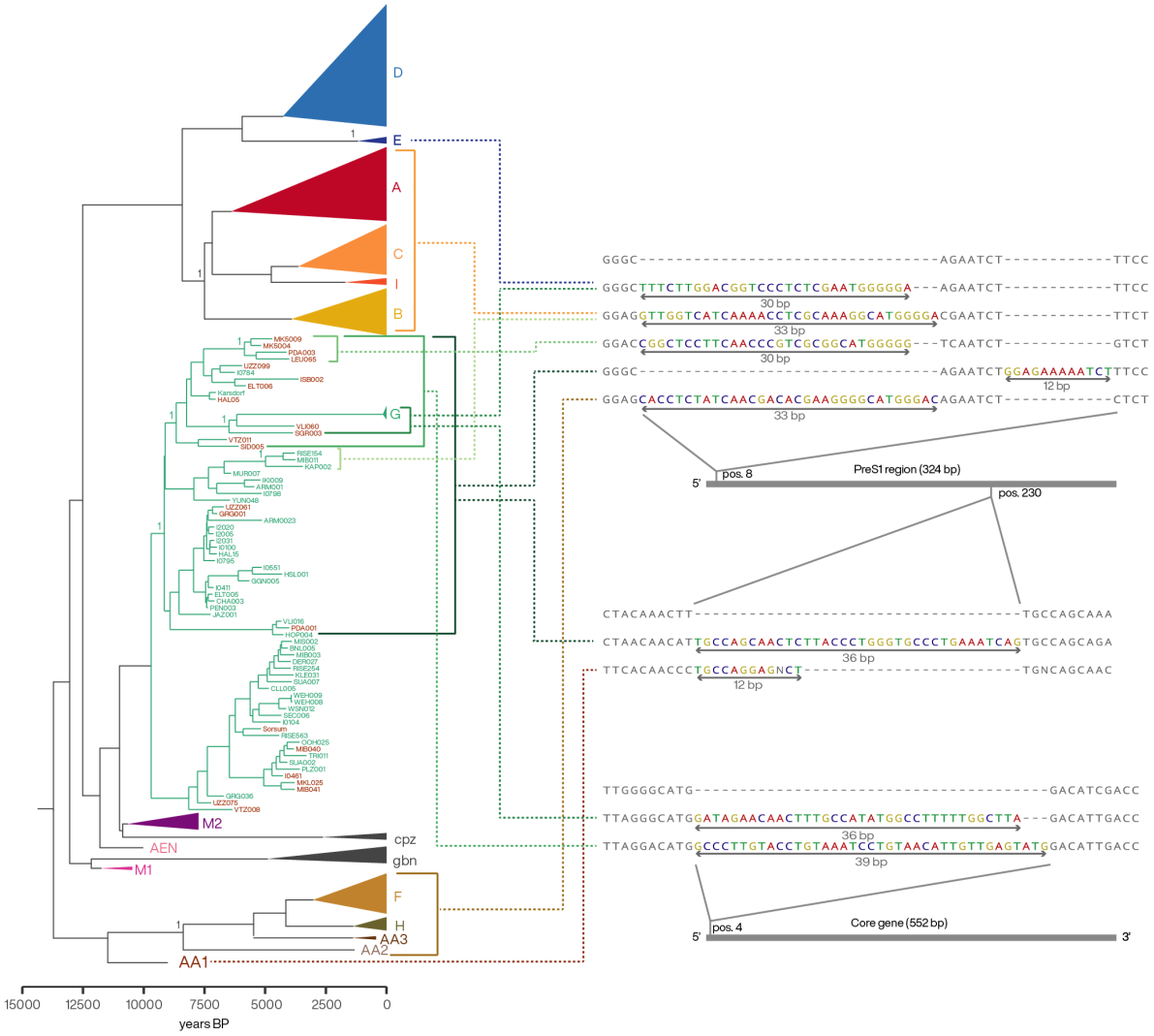

Fig. S8.
Insertions found in the core gene and PreS1 region of HBV genomes. For each genomic position in which insertions were found, an alignment of the main variants is shown. Insertions were aligned to facilitate visualization but might not represent homologous regions (they were masked for phylogenetic analyses). The first sequence corresponds to the short variant found in all genomes unless specified otherwise. Each insertion was found in entire monophyletic groups which are indicated by brackets on the time-calibrated phylogeny, together with their posterior support values. Genomes in the WENBA clade that were found in the context of mixed HBV infections are marked in brown.

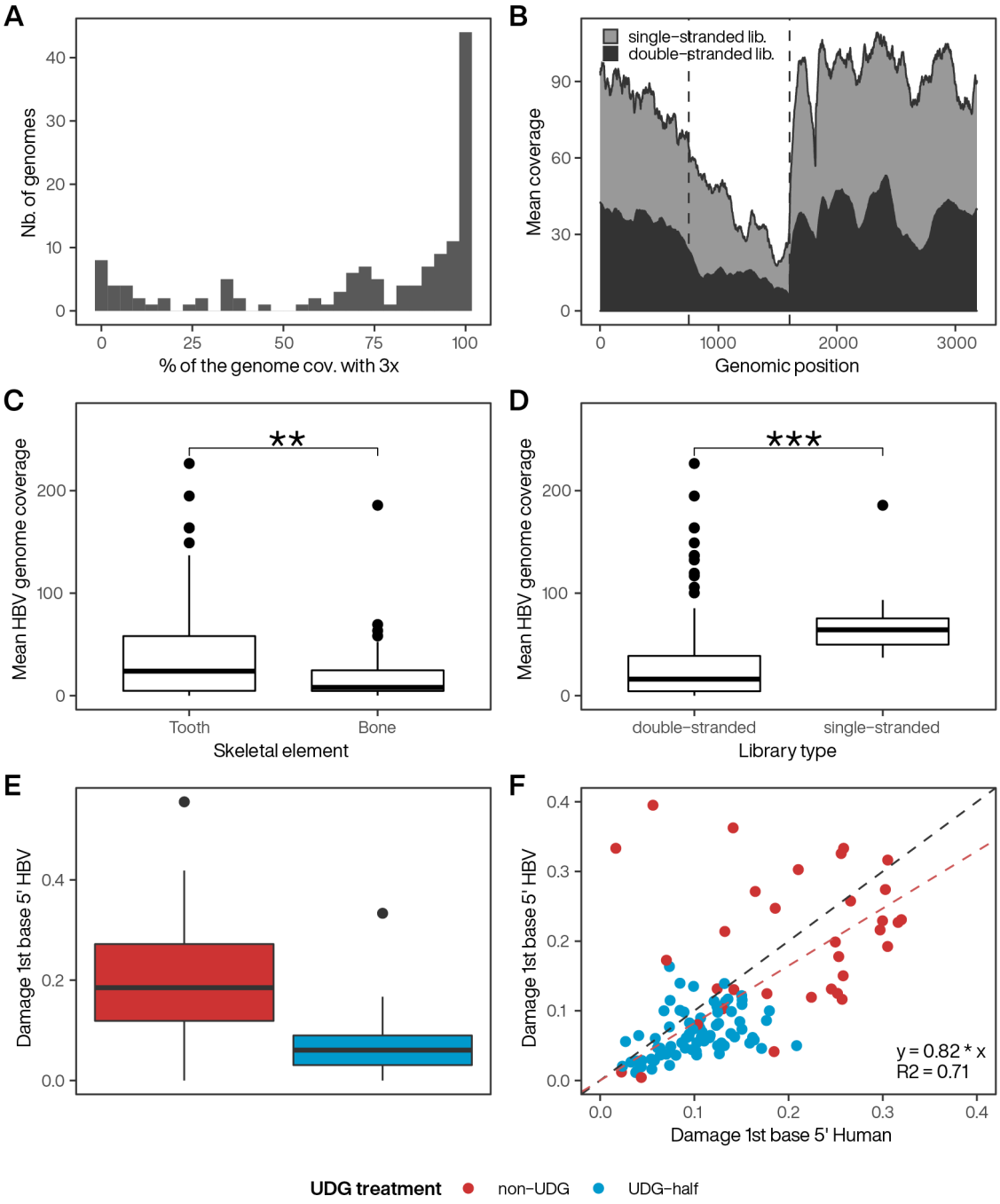

Fig. S9.
HBV genome coverage and damage statistics. (**A**) Histogram of the percentage of HBV genome recovered with > 3x coverage. (**B**) Sequencing coverage across the HBV genome averaged over all libraries. Dotted lines represent approximate boundaries of the single-stranded region of the genome. (**C**,**D**) Boxplots of the mean coverage of HBV genomes recovered from each library, depending on the skeletal element from which DNA was extracted and the library protocol employed. Significance levels of pairwise mean comparisons based on a combined mixed linear model are represented with the following codes: *p<=0.05, **p<=0.01, ***p<=0.001. (**E**) Boxplots of the frequency of damage-like substitutions on the 5' end of sequencing reads depending on the UDG treatment of libraries. (**F**) Scatter plot showing the correlation between the frequency of damage-like substitutions

in HBV vs. Human-mapping reads across sequenced libraries. The identity line (y=x) is represented in black, and the fitted regression line in red.

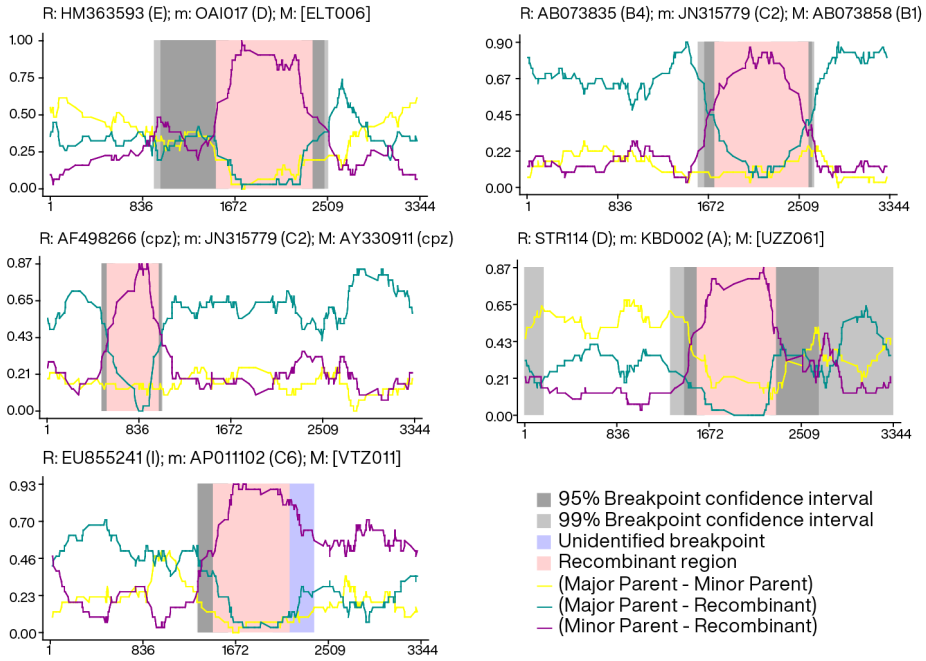

Fig. S10.
RDP plots showing evidence of recombination events. The x-axes refer to the position in the sequence alignment. The y-axes refer to the pairwise identity of sequence pairs (restricted to phylogenetically-informative sites), as indicated by the color of each line. For each plot we indicate the identity of the sequence triplet that was used to infer the recombination event (R: recombinant, m: minor parent, M: major parent). Sequences that were used to infer a recombination event without being identified as one of the parental sequences are indicated by square brackets. When the same event was detected in several sequences, we report only the triplet that provided the strongest signal.

Table S1.
Median estimates of HBV substitution rate and tMRCA obtained from the full dataset and different subsets of it.

Analysis	**tMRCA (years BP; 95%HPD)**	**Substitution rate (subst.site^{-1}.years^{-1}; 95%HPD**
full dataset	13518 (12119-15680)	1.54e-05 (1.34e-05-1.74e-05)
mixed infections excluded	13497 (12080-15707)	1.45e-05 (1.26e-05-1.64e-05)
recombination-free alignment	13588 (12161-15844)	1.47e-05 (1.29e-05-1.67e-05)
time-dependent rate	18710 (17170-20240)	from 1.79e-05 (1.66e-05-1.93e-05) to 8.7e-06 (7.9e-06-9.7e-06)

Table S2.
Results of low-coverage genome lineage assignments using EPA-ng.

ID	Country	Date	Taxonomic path	Assignment	LWR*
DOG007	Netherlands	9,374	HBV;Mesolithic 1	Mesolithic 1	0.81
TTK002	Tajikistan	8,222	HBV	Undetermined	0.93
UOO011	Russian Federation	8,050	HBV;Mesolithic 2	Mesolithic 2	1.00
UOO024	Russian Federation	8,050	HBV;Mesolithic 2	Mesolithic 2	0.98
SMH004	Germany	7,131	HBV;WENBA	WENBA	0.73
I2027	Germany	7,100	HBV;WENBA	WENBA	0.55
I2017	Germany	7,100	HBV;WENBA	WENBA	0.96
I2006	Germany	7,100	HBV;WENBA	WENBA	0.93
I2032	Germany	7,100	HBV;WENBA	WENBA	0.97
I0797	Germany	7,075	HBV;WENBA	WENBA	0.95
I0796	Germany	7,075	HBV;WENBA	WENBA	0.83
ADR001	Spain	7,073	HBV;WENBA	WENBA	0.99
VAR021	Bulgaria	6,425	HBV;WENBA	WENBA	0.84
I0163	Germany	6,350	HBV;WENBA	WENBA	0.87
I0162	Germany	6,350	HBV;WENBA	WENBA	0.93
ART005	Turkey	5,662	HBV;WENBA	WENBA	1.00
ART024	Turkey	5,374	HBV;WENBA	WENBA	0.98
I0554	Germany	5,150	HBV;WENBA	WENBA	1.00
I0111	Germany	4,290	HBV;WENBA	WENBA	0.98
QUE004	Germany	4,243	HBV;WENBA	WENBA	0.98
WSN010	Germany	4,135	HBV;WENBA	WENBA	0.99
NV3001	Russian Federation	3,970	HBV;WENBA	WENBA	0.98
KDC001	Russian Federation	3,813	HBV;A	A	0.99
ALA110	Turkey	3,800	HBV;A	A	0.92
KEL045	Lebanon	3,768	HBV;A	A	0.99
ALA098	Turkey	3,650	HBV;A	A	0.94
I0960	Russian Federation	2,586	HBV;A	A	0.85
CAA002	Cuba	1,572	HBV;Ancient American 2	Ancient American 2	0.94
I0972	Peru	725	HBV;F	F	0.99
HUA056	Peru	596	HBV;Ancient American 3	Ancient American 3	1.00
HUA063	Peru	596	HBV;Ancient American 3	Ancient American 3	0.96
SJN005	Mexico	417	HBV;H	H	1.00

*LWR: likelihood weight ratio

Table S3.
Results of lineage assignments of minor strains within mixed infections using EPA-ng.

ID	Country	Date (midrange estimate, years BP)	Lineage major strain	Assignment minor strain	LWR*
UZZ075	Italy (Sicily)	7,224	WENBA	WENBA	0.98
HAL05	Germany	7,042	WENBA	WENBA	0.73
GRG001	France	6,969	WENBA	WENBA	1.00
UZZ061	Italy (Sicily)	6,698	WENBA	WENBA	0.99
ELT006	Spain	5,800	WENBA	WENBA	0.94
Sorsum	Germany	5,170	WENBA	WENBA	0.13
I0461	Spain	4,270	WENBA	WENBA	0.78
LEU065	Germany	4,008	WENBA	WENBA	0.34
PDA001	Czech Republic	4,006	WENBA	WENBA	0.99
SGR003	Russian Federation	3,850	WENBA	WENBA	0.99
MKL025	Czech Republic	3,780	WENBA	WENBA	1.00
MIB041	Czech Republic	3,774	WENBA	WENBA	1.00

*LWR: likelihood weight ratio

Table S4.
Minimum and maximum genetic distances between modern genotypes and different groups of ancient strains, together with the results of mPTP classification.

Genotype	mPTP class	A	Ancient A	B	C	D	Ancient D	E	G	I	gbn	cpz	M1	AEN	M2	WENBA
A	1	0/6	2.2/8.4	7.6/10.8	8.1/10.4	8.8/11.3	7.8/10.5	8.2/10.3	10/11	7/8.4	9.7/11.9	9.4/10.8	8/10.3	8/9.2	7.9/10.3	7/11.6
Ancient A	1,2	2.2/8.4	0/7.2	6.8/10	6.5/9.1	7.1/10.5	5.6/9.3	7.5/9.2	8.2/10.3	5.4/8.1	8.6/11.1	8/9.9	6.5/8.6	6.2/8.1	6.1/8.4	5.5/10.7
B	3	7.6/10.8	6.8/10	0/7.2	7.8/11.3	9.2/11.4	8.5/11	9.2/11.3	11.2/12.1	8/10.2	9.6/11.5	9.6/11.2	8/10.6	8/9.3	8/10.5	6.9/11.8
C	4	8.1/10.4	6.5/9.1	7.8/11.3	0/7.8	9/11.4	8/10.6	8.8/10.9	11.1/12.6	6.4/8	9.3/11	8.7/10.9	7.1/9.6	6.9/8.2	7.4/9.4	7/11.4
D	5	8.8/11.3	7.1/10.5	9.2/11.4	9/11.4	0/6.4	1.2/6.2	6.8/8.9	10.7/11.9	9/10.5	10.2/12.3	9.9/11.4	8.7/10.8	7.4/8.8	7.5/10	6.4/11.3
Ancient D	5	7.8/10.5	5.6/9.3	8.5/11	8/10.6	1.2/6.2	0/4.4	6.3/7.8	10/11	7.8/9.6	9.4/11.7	8.5/10.4	7.4/9.5	6.5/7.8	6.4/8.8	5.2/10.6
E	6	8.2/10.3	7.5/9.2	9.2/11.3	8.8/10.9	6.8/8.9	6.3/7.8	0/2.3	10.3/10.6	9/9.6	9.9/10.8	8.8/9.9	8.2/8.9	7.6/7.9	7.1/8.1	5.7/10
G	7	10/11	8.2/10.3	11.2/12.1	11.1/12.6	10.7/11.9	10/11	10.3/10.6	0/0.3	9.8/10.2	11/12.5	10.6/11.1	10/10.5	7.8/7.9	8.7/9.4	7/11
I	4	7/8.4	5.4/8.1	8/10.2	6.4/8	9/10.5	7.8/9.6	9/9.6	9.8/10.2	0/3.3	9.7/10.5	9.4/9.9	7.5/8.3	7.2/7.6	7.1/7.9	6.4/10.4
gbn	8	9.7/11.9	8.6/11.1	9.6/11.5	9.3/11	10.2/12.3	9.4/11.7	9.9/10.8	11/12.5	9.7/10.5	0/9.3	8.9/10.2	5.6/7.5	5.5/7.3	5.9/9.2	6.6/11.3
cpz	9	9.4/10.8	8/9.9	9.6/11.2	8.7/10.9	9.9/11.4	8.5/10.4	8.8/9.9	10.6/11.1	9.4/9.9	8.9/10.2	0/5.4	6.5/7.8	6.2/6.5	5/6.2	5.2/9.6
M1	8	8/10.3	6.5/8.6	8/10.6	7.1/9.6	8.7/10.8	7.4/9.5	8.2/8.9	10/10.5	7.5/8.3	5.6/7.5	6.5/7.8	0/3.1	3.2/4.6	3/5.2	3.9/9.8
AEN	10	8/9.2	6.2/8.1	8/9.3	6.9/8.2	7.4/8.8	6.5/7.8	7.6/7.9	7.8/7.9	7.2/7.6	5.5/7.3	6.2/6.5	3.2/4.6	0/0	2.7/3.4	2.8/6.7
M2	11	7.9/10.3	6.1/8.4	8/10.5	7.4/9.4	7.5/10	6.4/8.8	7.1/8.1	8.7/9.4	7.1/7.9	5.9/9.2	5/6.2	3/5.2	2.7/3.4	0/2.6	1.9/7.7
WENBA	12-20	7/11.6	5.5/10.7	6.9/11.8	7/11.4	6.4/11.3	5.2/10.6	5.7/10	7/11	6.4/10.4	6.6/11.3	5.2/9.6	3.9/9.8	2.8/6.7	1.9/7.7	0/8.2

*M1/2: Mesolithic 1/2; AEN: Anatolian Early Neolithic; WENBA: Western-Eurasian Neolithic-to-Bronze-Age

Table S5.
Results of phylogenetic model selection using path sampling

tree prior	clock model	Bayes factor (log)*
Skyline coalescent with 5 time intervals	Relaxed lognormal	0
Birth-death skyline	Relaxed lognormal	-36
Skyline coalescent with 10 time intervals	Relaxed lognormal	-44
Exponential coalescent	Relaxed lognormal	-80
Constant coalescent	Relaxed lognormal	-153
Birth-death skyline	Strict	-431
Skyline coalescent with 10 time intervals	Strict	-433
Skyline coalescent with 5 time intervals	Strict	-435
Exponential coalescent	Strict	-491
Constant coalescent	Strict	-572

* The Bayes factors reported here are relative to the model yielding the highest marginal likelihood

The following 4 files (Data S1, Data S2, Data S3, Data S4) are too large to display in the thesis and have been burnt onto CD which accompanies this thesis

Data S1. (separate file)
Summary of contextual information for the individuals from which ancient HBV genomes were recovered in this study, together with details on library preparation and sequencing statistics, as well as on the characteristics of reconstructed HBV genomes.

Data S2. (separate file)
Plots used to identify mixed HBV infections in the studied individuals: distribution of genomic positions that were covered by at least 10 reads and exhibited clear heterozygosity-like signal, i.e. for which the major variant support was significantly below 90% and the second major variant support was significantly above 10%, after exclusion of damage-like variation. For each of these positions, we plot the major variant relative support with 95% CI. Genomic positions covered >10x are indicated with red bars.

Data S3. (separate file)
Damage plots: misincorporation rates observed at the 5' and 3' end of HBV-mapping reads (only for non-UDG and UDG-half libraries for which at least 100 HBV-mapping reads were recovered).

Data S4. (separate file)
HBV sequence alignments in fasta format, including the complete alignment and the masked alignment used for phylogenetic analyses, as well as complete and masked alignment of low-coverage genomes (consensus sequences obtained using a 1x-coverage threshold used for lineage assignments).

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7. Manuscript D

Using Y-chromosome capture enrichment to resolve haplogroup H2 shows new evidence for a two-path Neolithic expansion to Western Europe

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Abstract

Uniparentally-inherited markers on mitochondrial DNA (mtDNA) and the non-recombining regions of the Y chromosome (NRY), have been used for the past 30 years to investigate the history of humans from a maternal and paternal perspective.

Researchers have preferred mtDNA due to its abundance in the cells, and comparatively high substitution rate. Conversely, the NRY is less susceptible to back mutations and saturation, and is potentially more informative than mtDNA owing to its longer sequence length. However, due to comparatively poor NRY coverage via shotgun sequencing, and the relatively low and biased representation of Y-chromosome variants on capture assays such as the 1240k, ancient DNA studies often fail to utilize the unique perspective that the NRY can yield.

Here we introduce a new DNA enrichment assay, coined YMCA (Y-mappable capture assay), that targets the "mappable" regions of the NRY. We show that compared to low-coverage shotgun sequencing and 1240k capture, YMCA significantly improves the mean coverage and number of sites covered on the NRY, increasing the number of Y-haplogroup informative SNPs, and allowing for the identification of previously undiscovered variants.

To illustrate the power of YMCA, we show that the analysis of ancient Y-chromosome lineages can help to resolve Y-chromosomal haplogroups. As a case study, we focus on H2, a haplogroup associated with a critical event in European human history: the Neolithic transition. By disentangling the evolutionary history of this haplogroup, we further elucidate the two separate paths by which early farmers expanded from Anatolia and the Near East to western Europe.

aDNA, Y Chromosome, target enrichment, Neolithic expansion, uniparentally inherited marker

Introduction

Uniparentally inherited markers such as mtDNA and the NRY are an attractive source of information about the demographic history of a population due to the fact that their history can be represented by a simple evolutionary tree (Brown 1980; Jobling et al. 2003). Since the seminal studies of the 1980s (Cann et al. 1987; Torroni et al. 1984) and prior to the genomic era, much of the genetic history of humankind and the peopling of the world was inferred from uniparentally inherited mtDNA and NRY (e.g. Pakendorf and Stoneking 2005; Underhill et al. 2007; Kivisild 2017)

Due to the high copy number of mtDNA in the cells (Ingman and Gyllensten 2001), the short genome length (<17kb), and the relatively high substitution rate (Soares et al. 2009), mtDNA has been particularly well-studied, yielding an inexpensive and yet reliable source of information about the genetic variability of a population (Finnilä et al. 2001; Torroni et al. 2006; Posth et al. 2016).

Conversely, the mappable portion (the regions for which short reads, such as in ancient DNA studies, have been reliably mapped) of the NRY is much longer (~10,445kb) and presents only as single-copy in the cells of male individuals. The evolutionary substitution rate (in substitutions per site per year) was estimated to be up to two orders of magnitude lower for the NRY, e.g. $7.77 \times 10^{-10} - 8.93 \times 10^{-10}$ (Helgason et al. 2015) than for the entire mitogenome, e.g. $1.36 \times 10^{-8} - 1.95 \times 10^{-8}$ (Soares et al. 2009, though much debate surrounds estimating substitution rates (Scally & Durbin 2012). However, the greater genome length of the NRY, compared to the mtDNA, means that from these substitution rates, and for a single lineage, we still expect to observe a point mutation approximately every ~108 to ~123 years for the NRY, compared to between ~3094 to 4440 years for the entire mitogenome. Consequently, the NRY can contain more information about the paternal demographic history of a population and can be informative about male-biased population demographic changes, such as through male-driven migration (Karmin et al. 2015; Zeng at al. 2018; Olalde et al. 2019) or patrilocality (Deguilloux et al. 2013), so seeking insights into the paternal history of a population can be of critical importance.

When studying the demographic history of humans, aDNA has been shown to be an irreplaceable source of information. aDNA studies have revealed large-scale population movements and genetic turnover events in Western Eurasia (e.g. Fu et al. 2016; Allentoft et al. 2015; Haak et al. 2015; Olalde et al. 2018) that were otherwise impossible to recover from human genetic data of modern-day populations. Studies of the uniparentally inherited markers of ancient individuals have also yielded otherwise undetectable results, e.g. the loss of European mtDNA diversity following the repeopling of Europe after the last glacial maximum (Posth et al. 2016), or the decrease in and partial replacement of diversity of hunter-gatherer Y-chromosome lineages in eastern and central Europe following the Neolithic expansion (Bramanti et al. 2009; Haak et al 2010; Brandt et al. 2013; Lipson et al. 2017; Mathieson et al. 2018), followed by the loss of diversity of Neolithic Y-chromosomes lineages with the arrival of Steppe-like ancestry at the beginning of the 3rd millennium BCE (Allentoft et al. 2015; Haak et al. 2015; Olalde et al. 2018, 2019).

Researchers using aDNA data usually encounter problems related to sample quality, specifically a decrease of endogenous human DNA due to post-mortem DNA decay and environmental contamination (Higgins et al. 2015; Llamas et al. 2018). The Y chromosome

makes up <2% of the total DNA in male cells, meaning that if researchers wish to use shotgun (SG) sequencing to adequately cover enough informative sites on the single-copy NRY, then, even for samples with good DNA preservation, a substantial sequencing effort is required.

The development of targeted capture assays has allowed aDNA researchers to enrich specific sites and regions of the genome for sequencing, vastly improving the yield of human endogenous DNA from ancient samples (Fu et al. 2014; Mathieson et al. 2015). One such popular assay is the 1240k assay, which targets ~1.24M ancestry-informative sites on the human genome, of which ~32K represent a selection of known variants on the Y chromosome (based on an ISOGG list of informative Y-chromosomal SNPs as of 2013/14)(Mathieson et al. 2015). Of note, the commercially available version (myBaits Expert Human Affinities, Daicel Arbor Biosciences) contains an additional 46K Y-chromosomal SNPs identified by ISOGG to be variable in extant males.

This relatively low number of targeted Y-SNPs, compared to the number of currently known, informative Y-SNPs (as defined by ISOGG, n=73,163, or Yfull, n=173,801, https://isogg.org/tree; https://www.yfull.com), allows for basic Y haplogroup (YHG) assignments, but is heavily biased towards modern-day diversity and certain geographic regions. As a consequence, depending on the representation of particular Y-SNPs on the 1240k assay, the resulting YHG assignments can be of low and uneven resolution, while the target approach does not allow the detection of hidden and/or potentially extinct lineages in the human past.

To better study and understand the male history of human populations, we saw a need for a targeted assay that specifically enriches sequence data for sites on the NRY, without targeting only already well-known SNPs. To achieve this, we designed and implemented YMCA (Y-mappable capture assay), a tiled capture-assay for NRY sequence data that targets regions of the NRY for which short reads, typical in ancient DNA samples, are reliably mapped to the human genome, as defined by Poznik et al. (2013). A similar approach has been explored by two previous studies (Cruz-Dávalos et al. 2018; Petr et al. 2020). However, we avoid in-depth comparison with the probe set presented by Petr et al. which targets ~6.9 Mb was designed to substantially older samples, such as Denisovan and Neanderthal individuals, and hence the definition of “mappable” was far more conservative and stricter. Conversely, Cruz-Dávalos et al. also present a capture-enrichment approach designed for ancient human samples with low endogenous DNA. The reported ~8.9 Mb regions are almost completely included in our target regions (99.97%), and we show that the remaining ~1.5 Mb in our target regions still yield reliably mapped sites (see Supplementary Table S1.4).

Here we show that YMCA significantly improves the relative coverage of NRY sites when compared to shotgun sequencing, allowing for the enrichment of NRY sites for the same sequencing effort. We also show that YMCA significantly outperforms 1240k SNP assay sequencing in two ways. Empirically, we show that YMCA improves the number of NRY sites that are covered. We also show that, by considering the targeted NRY sites as defined by the associated bed files, that if we were to sequence a sample with high complexity to exhaustion, that YMCA has an improved potential resolution for Y-haplogroup assignment and the discovery of new diagnostic SNPs when compared to 1240k assay sequencing.

We highlight the improved performance obtained via YMCA by analysing the Y-chromosomal haplogroup H2 (H-P96), a low-frequency YHG that is associated with early farmers during the Neolithic transition in Western Eurasia. We curated a data set of 46 previously published individuals (45 ancient and 1 modern), and 49 newly YMCA-sequenced individuals (all ancient). We show that our current understanding of H2, which is based largely on modern H2 samples (n=20), is inconsistent with the ancient diversity of our H2 individuals. In resolving this ancient haplogroup, we can show two distinct migration paths along the Mediterranean and Danube for Neolithic groups from Anatolia to Western Europe, ultimately resulting in the Mediterranean-derived groups also reaching the Atlantic Archipelago/Britain and Ireland/British Isles.

Results and Discussion

Validating the performance of YMCA

To evaluate the performance of our new NRY-Capture assay (YMCA), we calculated the empirical fold-increase in endogenous human DNA for a range of samples with varying levels of preservation. We chose samples from the same site (Leubingen, Germany) to avoid the effects of too many environmental variables, for which we had shotgun, 1240k capture and YMCA sequence data (see Table S3). We then compared the empirical performance of YMCA against standard shotgun sequencing and 1240k capture on the same libraries by inspecting the number of NRY sites covered, as well as the number of ISOGG SNPs covered at least once for each library type. We account for sample quality and input sequencing effort by filtering for only human endogenous reads, and then normalising the number of sites/SNPs covered per five million endogenous reads.

We observed a significant fold-increase in the amount of endogenous human DNA when comparing shotgun sequencing to YMCA (see Figure S1), which we refer to as “enrichment” from here on. We found that enrichment diminished as the preservation of the sample increased, i.e. for samples with higher starting endogenous DNA % the effect of the enrichment was reduced, but still significant.

We observed a significant mean fold increase of ~15.2X in the number of NRY sites covered by YMCA captured libraries when compared to shotgun sequencing ($p=5.5 \times 10^{-7}$), and ~1.84X when compared to 1240k sequencing ($p=8.8 \times 10^{-12}$), showing that YMCA covers on average more NRY sites than both shotgun and 1240k sequencing. This also indicated that, since we covered on average 15.2 times as many ISOGG SNPs per five million reads for SG sequencing, we would need to sequence ~76 million reads to cover the same number of NRY sites for shotgun sequencing compared to only 5 million reads for YMCA.

Interestingly, we also found mean fold increase of ~4.36X in the number of ISOGG SNPs covered at least once with YMCA captured libraries when compared to 1240k sequencing ($p=9.0 \times 10^{-14}$). This indicated that, for the same sequencing effort, YMCA also covers more informative SNPs.

We also found that the fold-increase in the number of NRY sites that we covered, and the endogenous DNA percentage for shotgun and 1240k sequencing were uncorrelated (p=0.976 and p=0.617), and that the number of ISOGG SNPs covered and the endogenous DNA percentage for 1240k sequencing were uncorrelated (p=0.1825) indicating that our results are not dependent on the relative abundance of retrievable human DNA in the sample. Hence, we found that, although the SNPs covered on the Y chromosome are an added bonus when using the 1240k assay, as it is primarily used for analysing the autosomal genome of male and female individuals, YMCA is clearly a significant improvement if researchers wish to efficiently and thoroughly investigate the non-recombining portion of the Y chromosome.

We then compared the percentage of haplogroup-informative SNPs on the ISOGG SNP list v14.8, that are also included in the 1240k assay, and YMCA, according to their respective bed files. This comparison will be particularly powerful as YMCA and the 1240k assay are based on the same technology, and captured via identical lab protocols. The 1240k assay targets 24.44% of the currently listed ISOGG SNPs, whereas the YMCA targets 90.01% (Figure 2). Note that the remaining 9.99% of ISSOG SNPs exist in regions of the NRY which are considered "unmappable" for short reads common in ancient DNA. Since each of the sites in the1240k assay is targeted by two probes (allele and alternate allele) and two 52bp probes on either side of the variant, additional sites flanking the "targeted" sites can also be recovered from the mapped reads. Hence, we also allow a window of 120bp (60bp on either side) for each SNP on the 1240k assay, which is a reasonable average read length for aDNA. For this 1240k+120bp list of sites the percentage of targeted ISOGG SNPs increases to 45.34%, but this also illustrates that the 1240k SNP assay is fundamentally limited by the total number of informative Y chromosome SNPs included. This significant increase in ISOGG targeted SNPs would also explain why, for the same sequencing effort, YMCA covers more ISOGG SNPs.

Additionally, recovering as much of the NRY as possible is of critical importance, especially when researchers are interested in looking for new variants on the Y chromosome, or uncovering past diversity that might no longer exist. When comparing the raw number of sites targeted by the 1240k assay to YMCA, we observed that the 1240k capture assay potentially targets a total of 32,670 sites, which is approximately 0.31% of the number of sites targeted by YMCA (~10,445K). However, if one is to include a window of 120bp around each SNP again, then the 1240k assay potentially targets ~3,953K sites or 37.82% of the number of sites one can potentially analyse using our YMCA. Hence, YMCA is a predictably better tool for exploring the NRY for new ancestry informative SNPs.

We were also interested in comparing the potential resolution to which YHG assignments can be made, given the available ISOGGs SNPs targeted by YMCA and 1240k. We also found that the resolution of a YHG call cannot be improved, even when including a 120bp tiling window around the ~32K Y-SNPs of the 1240k assay, according to the ISOGG SNPs occurring in the respective bed files. This holds true both for dominant YHGs today and in particular for those that are associated with known ancient populations, but that have significantly reduced in frequency in modern populations, and which are not well covered for diagnostic SNPs on the 1240k assay.

We often observe low resolution in haplogroups such as the early hunter-gatherer haplogroup C-V20 (Fu et al. 2016), and the Neolithic expansion-associated G-Z38202 (Lacan et al. 2011) and H-P96, for which the Y-SNPs of the 1240k assay target 0.8%, 0% and 13% of

the associated ISOGG SNPs, respectively (we include SNPs within three branches downstream of each terminal SNP). If we include a 120bp window, then these percentages increase to a more respectable 32.5%, 31.2% and 36.2%, which are still much lower than the 89.6%, 90.6% and 95.2% of SNPs targeted by YMCA (see Figure 2). In addition, poor theoretical coverage for YHGs which are thought to be present in early human population movements, but which remain relatively prevalent in modern populations, can still be an issue for sites on the 1240k assay. For example, Q-M3, which is associated with the initial peopling of the Americas (Ruiz-Linares et al. 1999) has only 11.9% (33.5% if a 60bp window is included) of the relevant diagnostic SNPs covered, compared to 92% for YMCA.

To summarize, YMCA enriches the relative proportion of reads mapping to the NRY, when compared to shotgun sequencing and the 1240k assay. YMCA also targets more than 2.5 times as many sites on the NRY than the 1240k assay, allowing for the detection of new diagnostic SNPs. Critically however, YMCA targets SNPs which are already known to be informative, but the 1240k assay cannot target.

Application of YMCA to YHG H2 as a case study

Through routine application of SG sequencing for sample screening, followed by 1240k capture for suitable samples in our lab, we were able to explore the performance of the new YMCA on a range of YHG in ancient male individuals. Here, we showcase an example of YHG H-P96, for which the resolution of the evolutionary tree is still unclear due to the scarcity of data and low frequency in modern-day populations. Judging from our current ancient DNA record, it appears that YHG H was more common in the past, in particular among males that were associated with the spread of farming across Western Eurasia during the Neolithisation. As a result, we can show that aDNA research, and in particular high-resolution typing of YHG, can help elucidate the evolutionary relationship of Y chromosome lineages past and present.

The YHG H (H-L901) is thought to have formed in South Asia approximately ~48 kya (Sengupta et al. 2006). Three subsequent sub-haplogroups, H1 (H-M69), H2 (H-P96) and H3 (H-Z5857), appear to have quickly formed over the following four thousand years. H1 and H3 have estimated formation times of ~44.3 kya, however, H2 is estimated to have formed slightly earlier at ~45.6 kya [https://www.yfull.com].

H1 and H3 are still found in frequencies as high as 20% in South Asia (Rai et. al. 2012), but in extremely low frequencies in Europe, with H1 only being found associated with the spread of the Romani people ~900 ya. Conversely, H2 has been present in Western Eurasia since at least 10 kya (Lazaridis et al. 2016), and is strongly linked with the spread of agriculture (Hofmanová et al. 2016; Rivollat et al. 2020), but is found at no higher than 0.2% frequency in modern-day western European populations. In contrast, H2 was more common in Neolithic groups, and has been found to have constituted between 1.5% and 9% of the observed Y haplogroups, with the exception of the highly related samples from Rivollat et al. 2020, for which H2 was ~30% (Brunel et al. 2020, Haak et al. 2015, Mathieson et al. 2015, Lazaridis et al. 2016, Lipson et al. 2017, Olalde et al. 2019, Rivollat et al. 2020, Skourtanioti et al. 2020).

With the arrival of Steppe-related ancestry ~5 kya, incoming YHGs such as R1a and R1b would largely replace many of the older, Neolithic YHGs, such as G2, T1a, and H2 (Haak et al. 2015), and although H2 was never found in particularly high frequencies among Neolithic individuals,

we expect that its diversity was also greatly reduced, and many sub-lineages were potentially lost altogether.

To test whether our YMCA could improve the haplotyping quality to a point which would allow us to also draw phylogeographic inferences, we made use of newly collated collection of prehistoric ancient human DNA data and selected individuals, who were tentatively assigned to YHG H2. We generated new data for n=25 individuals, and merged this with n=71 published Y-chromosomal genomes (see Tables S2 and S3). While H2 is commonly found alongside the more dominant Neolithic YHG G2a (G-Z38302) (Lipson et al. 2017, Hofmanova et al. 2016), it is precisely the low frequency of H2 which is of interest here. The relative scarcity of H2 individuals, especially compared to the relatively high frequency of the accompanying G2a individuals, allows us to better track the 'genealogical history' and thus potential dispersal routes as we would expect a stronger effect of lineage sorting and therefore a higher chance of observing geographic patterns. In this particular case, we could trace expanding Neolithic farmers from Anatolia to Western Europe through the use of unique markers associated with H2 individuals and test whether we can genetically discern the proposed so-called "Danubian or inland'' and "Mediterranean'' routes of the Neolithic expansion (Price 2009), which had recently also found support by genomic signals from the nuclear genome (Rivollat et al. 2020).

Unfortunately, we found that the H2 subsection of the evolutionary tree for the Y chromosome is currently poorly understood (due to the scarcity of modern samples of H2 individuals and the relative rarity of ancient H2 individuals), and, in many cases, inconsistent with a tree-like history for almost all of the published and unpublished ancient samples. In all but one case that we found that H2 individuals carry a mixture of derived SNPs from two bifurcated clades in the current ISSOG topology, such as from H2a1 and H2b1. Encouraged by the performance of the YMCA presented above, we thus analysed further H2 individuals in an attempt to resolve the branching pattern of this lineage.

For a non-recombining portion of DNA, the evolutionary history is expected to follow a tree-like structure, and therefore hybrids of sibling haplogroups (such as between ISOGG H2a, H2b1 and H2c1a) are impossible. To try and find a better resolved evolutionary history for these individuals, we constructed a maximum likelihood (ML) phylogenetic tree using IQ-TREE (see Methods). We identified two major clades from the ML tree (see Figure 3A), and denoted these two tentatively named clades H2m (blue clade) and H2d (green clade). With respect to the current ISOGG nomenclature, we note that H2m appears to be defined by a mix of H2, H2a, H2a1~ and H2c1a~ SNPs (see Table S6). H2d appears to be defined by two H2b1 SNPs, and four additional SNPs which were previously undetected (see Table S7). Hence, it could be that H2d is simply derived from the basal H2 group, but with a few private mutations. However, we also note that H2d contained a sub-clade containing individuals from Turkey (ART) and Germany (DER) which were uniquely defined by a further 10 SNPs associated with H2b1, potentially indicating further sub-structure (see Figure S4).

Based on our extended set of diagnostic SNPs, we were able to assign n=58 of our samples to either one of these two sub-clades, or (basal) H2* (due to low coverage), even for samples that did not meet minimum coverage requirements to be included in the ML tree, which also provides bootstrap support for individual clades (Figure 3A). Finally, we also had three

individuals who were not derived for any of these additional SNPs, and were ancestral for many of the H2 SNPs (denoted basal, n=3).

When we plotted all of the samples in our study on a map of Europe, a phylogeographic pattern clearly emerged (Figure 3B. The H2d individuals are all found along the so-called inland/Danubian route into central Europe, and all but one of the H2m individuals are found along the so-called Mediterranean route into Western Europe, the Iberian Peninsula and ultimately, Ireland. The solitary H2m individual (LEU019) found in central Germany is dated to the Late Neolithic/Early Bronze Age context, postdating the Neolithic expansion by 2000-3000 years. Archaeological and mtDNA evidence of an eastward expansion of Middle/Late Neolithic groups such as Michelsberg (Jeunesse 1998; Küßner 2016; Beau et al. 2017) could potentially explain this single geographically outlying observation.

Due to the incomplete and varying coverage of our ancient samples, we were unable to produce a reliable calibrated tree for divergence time estimates using the radiocarbon dates of ancient samples as tip dates. Instead we estimated the time since the most recent common ancestor (TMRCA) for each pair of individuals to investigate the split times for our newly identified H2 clades (see Methods and Figure S2). First, we calibrated our relative substitution rate so that we estimated a mean TMRCA of ~161.3 kya for haplogroup A0 with all other haplogroups (see Figure S3). Using this calibrated substitution rate, we estimated a TMRCA for haplogroup A1 of ~133.2 kya, and a TMRCA of ~48 kya for haplogroup HIJK, which are extremely close to the current estimates of ~133.4 kya and ~48.5 kya respectively [https://www.yfull.com]. Our estimated TMRCA for H2 was ~24.1 kya, which is slightly older than the current estimate of ~17.1 kya, which could be explained by our extremely limited access (only one) to high-coverage modern H2 samples, and our increased number of ancient samples [https://www.yfull.com].

We found that the estimated TMRCA for H2d and H2m was ~15.4 kya. We also found that H2m and H2d had estimated TMRCAs of ~11.8 and ~11.9 kya (see Figure 4). We note, however, that even though the associated error bars are wider due to fewer overlapping SNPs, the mean estimates are still relatively consistent. These estimates, plus the fact that H2d and H2m individuals are found in Anatolia and the Levant, show that H2 diversity most likely existed in Near-Easterner hunter-gatherers before the establishment of agriculture and animal husbandry and likely also in early farmers, and subsequently spread via the Neolithic expansion into Central and Western Europe.

Identifying diagnostic SNPs for improved YHG H2 resolution

Having used YMCA to identify two novel subclades of H2, we also aimed to identify which SNPs are diagnostic for these subclades, when compared to the human genome reference hs37d5 (see Tables S5-S7). To do this we looked for segregating sites with the following properties: (1) no individual from the ingroup is ancestral at the site, (2) more than one individual from the ingroup is covered at the site, (3) no individual in the outgroup is derived at the site, and (4) more than one individual in the outgroup is covered at the site. We also restricted the search for “new” SNPs to substitutions that are not C->T or G->A, and thus are less likely to be the result of ancient DNA damage, however we included variants that are C->T or G->A in our results if they are previously-discovered SNPs in the ISOGG or YFull databases. Note, that when we report that x/N samples in a group are derived for some SNP,

this means that the remaining N-x samples are not covered at this position, and we have simply recorded a “missing” base read (Supplementary Tables S5-S7).

We identified 312 potential diagnostic SNPs for the sub-haplogroups/branches in Figure 3 defined as H2 (all samples), H2d (green) and H2m (blue). Encouragingly, we found that out of the 312 diagnostic SNPs that we identified, 258 (80.1%) are already found to be H2 associated (H2-P96 or more derived) on either the ISOGG list, or on the YFull SNP list. We found only two previously discovered SNPs (0.31%), which were not associated with H2: a C->T SNP associated with R1a1~ and R1a1a~ (found in 17/31 H2 samples). It is unlikely that the C->T substitution is due to damage since 17/31 samples have this substitution. Furthermore, we also found that for our ancient H2 individuals (except for one modern French H2 individual), we were able to find 110 of the 134 known, basal H2 SNPs.

The remaining 62 newly discovered SNPs for the varying sub-haplogroups listed above represent either undiscovered diagnostic SNPs, or potentially lost H2 diversity (Supplementary Tables S5-S7). However, for several of our newly discovered SNPs, such as an A->G substitution at site 8611196 (found in 20/31 of our H2 samples), we find overwhelming evidence for new, true diagnostic SNPs (see Table S5).

Our ability to detect these distinct H2 sub-haplogroups, and hence our ability to further elucidate and estimate the divergence times for an informative Y-haplogroup during the Neolithic expansion, is made possible only due to the increased coverage, and the increased number of sites we were able to target with YMCA (when compared to SG or 1240k sequencing).

Discussion

The analysis of the Y-chromosomal history of populations can be of significant importance to the understanding of population histories. To this end we advocate for the adoption of targeted sequencing strategies for ancient Y-chromosomal sequence data. Our focused study highlighted the improved coverage and number of SNPs that are attainable when using YMCA, when compared to SG or 1240k sequencing, for the same amount of sequencing effort, accounting for endogenous human DNA content.

Targeted endogenous human DNA enrichment is of critical importance to overcome poor sample preservation in ancient DNA studies. We have shown that the Y-SNPs of the 1240k assay ascertained from modern-day males, simply do not cover enough of the diagnostic SNPs on the NRY for reliable Y-haplogroup assignment, especially in the case of haplogroups that predate modern diversity, highlighting a need for targeting contiguous regions in favour of an updated “Y-chromosome SNP panel”. YMCA can be applied to the same libraries that are used for other captures and require no additional extractions or library preparations. While it is certainly possible to combine YMCA with other captures assays (which we have not attempted), we argue that a bespoke YMCA of selected male samples in a directed study might outweigh that of a routine combined application (to male and female samples) with additional sequencing effort.

We were also able to show, through a deeper analysis of H2 (H-P96), that the current understanding of ancient H2 diversity is incompatible with a tree-like history (which must be true for NRY history), and that a resolution of this diversity leads to further support for the two paths of the Neolithic expansion from the Near East into Europe; an observation that would not have been possible without the improved resolution offered by YMCA. We foresee future applications in the study of Y-chromosomal sub-structure in Eurasian hunter-gatherers (within I2a, I2b or C1a diversity) or to better characterise the R1a/R1b diversification of Bronze Age Western Eurasia, Central and South Asia.

Materials and Methods

Data

Note that for Y-haplogroup assignment, tree building and SNP identification, we use a mix of shotgun, 1240k, and YMCA capture sequencing runs. However, to estimate the TMRCA, we use only shotgun and YMCA data as they do not target known segregating sites (which would upwardly bias the substitution rate for samples with 1240k capture compared to those with shotgun and YMCA data only). Previously published samples were selected from published data with "H2" designated for Y haplogroups (Mathieson et al. 2015; Lazaridis et al. 2016; Lipson et al. 2017; Olalde et al. 2018, 2019; Narasimhan et al. 2019; Brunel et al. 2020; Antonio et al. 2019; Cassidy et al. 2020; Fernandes et al. 2020; Rivollat et al. 2020; Skourtanioti et al. 2020).

Contamination quality filtering

To screen our samples for contamination, we consider the heterozygosity for sites on the NRY as our in-house samples are all merged and filtered for sites on the Y-chromosome only. We measured heterozygosity (the proportion of sites with more one than one type of base read per site) for a pileup of the quality filtered reads. We found that 45 of our 47 samples had less than 0.1%, with the remaining 2 samples 1% and 1.85% heterozygous sites. However, we were also confident in the quality of our samples as H2 is a very rare modern haplogroup, with only 19 individuals being downstream of H2-P96 on YFull at the time of this publication. Hence, if any of our samples had been contaminated by a *male* source, it would be readily noticeable in bam pileups as derived alleles for another haplogroup, which means these samples would not have been identified as H2, and hence would not be in the study.

Method of Y Haplogroup Assignment

To assign Y haplogroups to samples we follow a partially-automated process. We begin by taking pre-prepared (i.e. trimmed, merged, deduplicated, quality-filtered) bam files, and, for each bam file, creating a pileup of every site that was covered using the *pileup* function in the Rsamtools library for the R statistical software package (Morgan 2020). We then filter this pileup of SNPs found on the ISOGG list [https://isogg.org/tree]], and then for each SNP we calculate and record the number of derived and ancestral SNP calls, the form of the ancestral and derived SNPs, and the difference (defined as the number of derived minus the number of ancestral SNP calls). Note that a positive difference indicates evidence for the ancestral form of the associated ISOGG SNP, and a negative difference indicates the converse. Recording the form of the called SNPs (i.e. C to T or G to A transitions), allows us to identify where DNA damage could have caused us to infer false calls.

We return two CSVs: one CSV of only ISOGG SNPs with positive differences (for ease of reading the easiest path from root to terminal SNP), and a second CSV of all SNPs (negative or positive) allowing us to double check potentially spurious SNPs (say to check to see if more basal branches from our terminal branch are not just missing, i.e. not covered, but also not associated with negative differences). This second CSV also allows us to discover when some SNPs are derived and some are ancestral for the same branch, indicating a transitional form of the basal haplogroup.
Finally, in cases where we are uncertain of the dependability of a call (say a C to T transition with only one read), we also manually inspect where the site falls on the associated read(s), placing increased trust in SNP calls originating further from the terminal ends of a read.

Comparing the Performance of our Y-capture Assay

When comparing the empirical performance of our Y-capture assay to both shotgun and 1240k sequencing, we took libraries for which shotgun, 1240k and Y-capture sequencing had all been performed. All samples were prepared and analysed using the same methods and parameters values as for the main data set.
To compare the theoretical performance of YMCA against the 1240k assay, we downloaded the ISOGG SNP list v15.64. We then took the bedfiles for the NRY and 1240k assay, and found which sites overlapped with the SNPs listed on the ISOGG SNP list.
When comparing empirical data performance for shotgun, 1240k and YMCA data, we included only samples that had shotgun endogenous DNA % greater than 0.1%, had sequencing results for shotgun, 1240k and YMCA sequencing and normalized the number of SNPs covered by the number of reads mapping to the human reference (hs37d5) after quality filtering. We did this to avoid any potential bias from sample quality or sequencing effort.

Phylogenetic Tree Reconstruction

We began by taking pileups of each bam file, and performing the following quality filters for calling a consensus alignment; for each sequence we considered only sites for which we had at least two reads, with a minimum allele frequency less than 10%, and called the majority allele. We then took the aligned consensus sequences, and kept only samples for which at least 1,100 segregating sites were covered, and then filtered sites for which more than at least one sample was covered. We selected a lower bound of 1,100 segregating sites by varying this value, and inspecting bootstrap node support values. A minimum bootstrap support for major cladal splits of 80% was required.
We also included high-coverage samples from the 1000 Genomes projects (1000 Genomes Project Consortium 2015) from Y-haplogroups A, H1, H2 and H3, as well as one ancient H1 sample (Narasimhan et al. 2019) as outgroups.
We performed DNA substitution model selection using ModelFinder (Kalyaanamoorthy et al. 2017) and selected the TVM model as it had the minimum Bayesian information criterion value. We found a maximum likelihood tree using IQ-TREE v.1.5.5 (Nguyen et al. 2015).

Declarations

Funding

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Conflicts of interest/Competing interests

The authors declare no competing or conflicts of interests.

Availability of Data and Material

Data generated for this study can be found at the European Nucleotide Archive under the study accession number PRJEB45741.

Code Availability

All R scripts can be obtained by contacting the corresponding authors.

Author Contributions

ABR, WH, JC and AH initiated and led the study. LP, SP, AC, MR, VVM, GUN, ES, MvdL, MA, KB, YB, MFD, MD, YSE, ME, MFr, MFu, SF, EG, AHa, SH, MK, MM, RÖ, SRe, SRo, DCSG, JSD, PWS, CRdTM, KAY, CP participated in the laboratory work, sample management, gathering of contextual information and organized the sample collection for genetic analyses. ABR performed the statistical analyses. All authors contributed to the interpretation of the data. ABR wrote the first version of the manuscript which was edited by WH, LP, SP, AHe and CP and all authors contributed to its improvement.

Animal Research (Ethics)

Not applicable.

Consent to Participate (Ethics)

Not applicable.

Consent to Publish (Ethics)

Not applicable.

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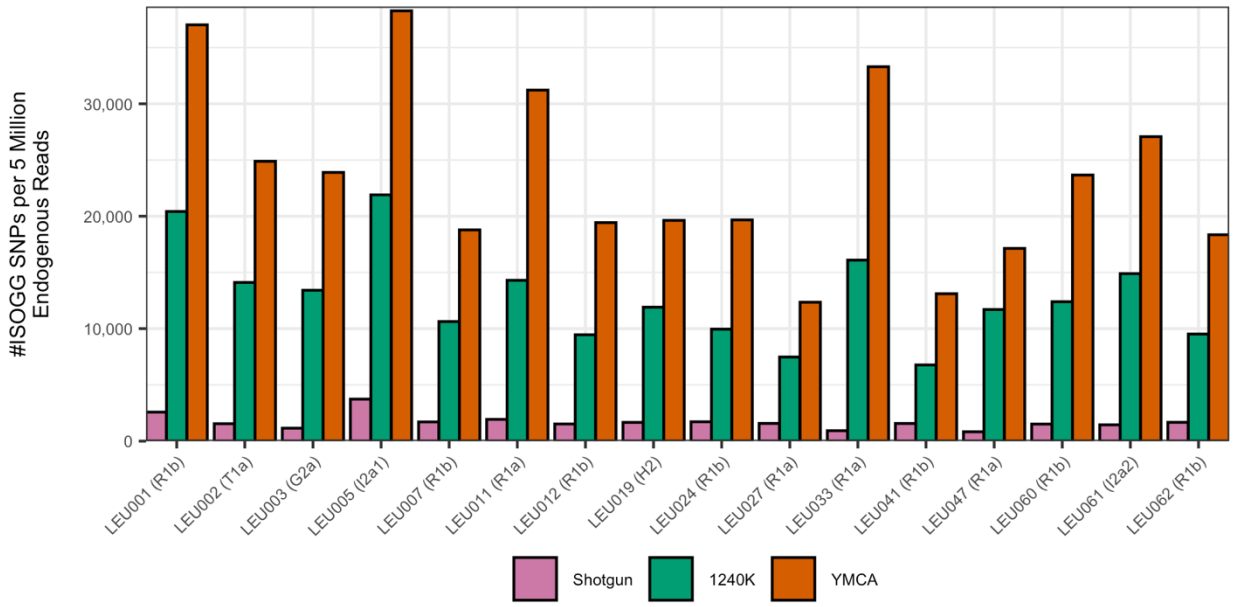

Figure 1: *The number of ISOGG SNPs covered per 5M quality-filtered mapped reads (y-axis) for the same libraries (x-axis) for shotgun, 1240k and YMCA sequencing (colours).*

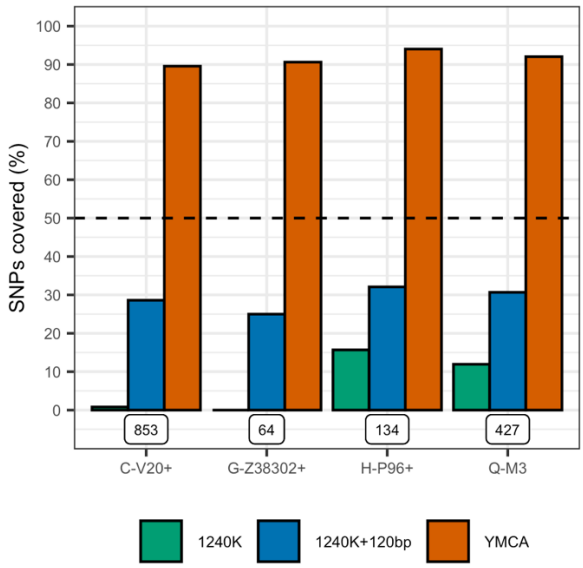

Figure 2: *The percentage of SNPs (y-axis) covered (up to three branches downstream) for four Y-chromosome haplogroups (x-axis) associated with ancient populations. Colours indicate assay SNPs targeted for 1240k (green), 1240k with a 120bp window (blue) and our YMCA (orange). The dashed black line indicates at least half of the SNPs are represented, and the total number of targetable SNPs is given below each group.*

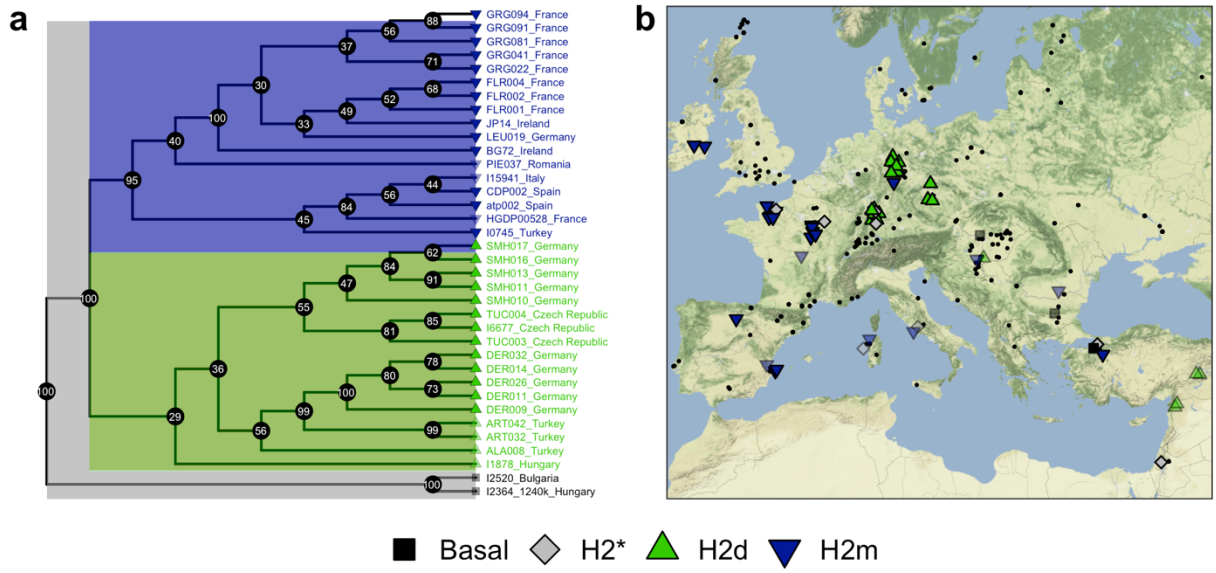

Figure 3: *(A) phylogenetic relationships (no branch length units) and (B) H2 sample locations. Shapes and colours indicate the two major clades inferred from the phylogenetic tree. Symbol shading indicates early to late Neolithic (solid) or post-Neolithic (transparent). Black dots indicate all non-H2 Neolithic individuals from Freeman2020 to indicate H2 sampling prevalence. Stars in haplogroup assignments in (a) indicate a lack of resolution to assign samples (not used in the ML tree) to downstream sub-haplogroups.*

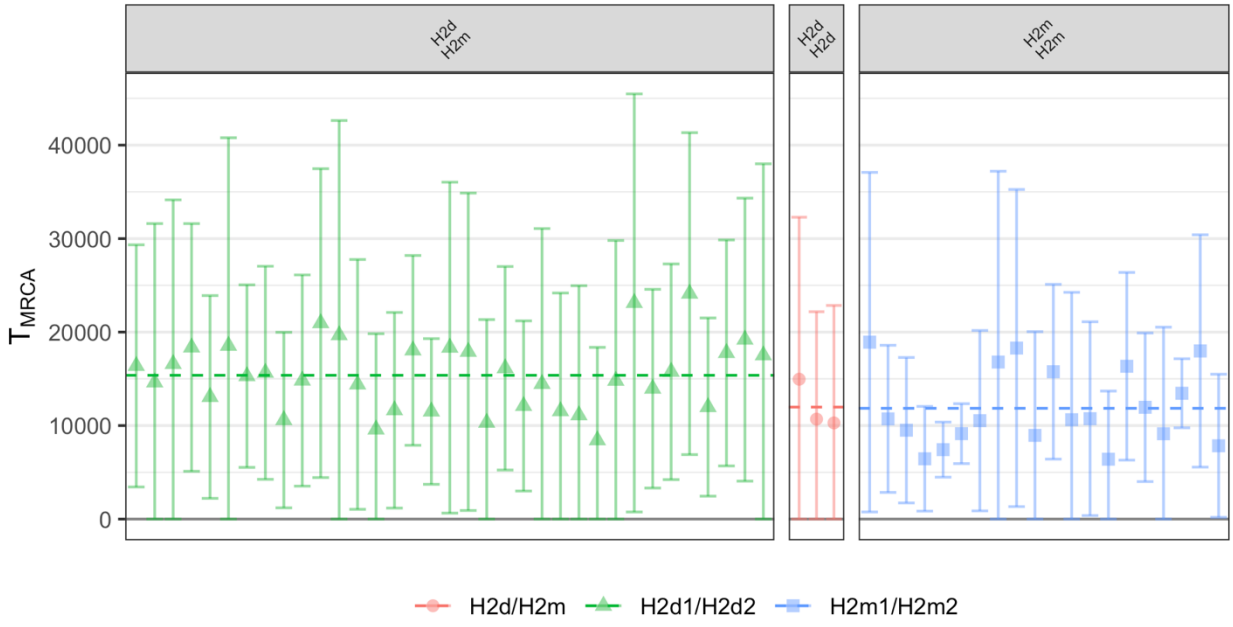

Figure 4: *Estimated time since most recent common ancestor (T_{MRCA}) (y-axis) for each H2 subgroup with each other (facets), calibrated by the split time of ~163 kya of haplogroup A0 with all other Y haplogroups. All pairwise calculations are filtered to exclude individuals from the same sampling site. The dashed line indicates the mean estimate, and error bars indicate 95% confidence intervals for individual observations.*

Materials and Methods

Design of the YMCA Probe Set

For targeted DNA enrichment probes were designed on the basis of callable regions on the Y-chromosome as previously determined by Poznik et al. (2013). The probes were designed with a 4bp tiling and a length of 52 bp with an additional 8bp linker sequence (CACTGCGG) as described in Fu et al. (2013). Duplicated probes were removed. This resulted in 2,611,534 unique probe sequences. This probe set was spread on three Agilent one-million feature SureSelect DNA Capture Arrays. The capacity of the three arrays was filled by randomly duplicating probes from the probe set. The arrays were turned into an in-solution DNA capture library as described in Fu et al. (2013).

Human genome enrichment and sequencing

113 petrous bones and 52 teeth were processed in the ancient DNA laboratory at the Max Planck Institute for the Science of Human History in Jena, Germany. Upon introduction into the clean room, samples were wiped with 10% bleach and irradiated with ultraviolet light for fifteen minutes on each side. Petrous bones were either sampled by cutting them in half before drilling out bone powder from the dense portion (Pinhasi et al. 2015) or by keeping them intact and drilling into the dense portion from the outside. Teeth were sampled by removing the crown and drilling into the pulp chamber to produce bone powder. Resulting bone powder (50-100mg) was placed in 2mL Biopure tubes and stored until DNA extraction.

To the Biopure tubes containing 50-100mg bone powder one mL of extraction buffer made up of 0.9mL 0.5M EDTA, 0.025mL 0.25mg/mL Proteinase K and 0.075mL UV HPLC-water was added. The resulting mixture was incubated at 37^{o}C for 20 hours under constant rotation. Following incubation, Biopure tubes were centrifuged at 18500 relative centrifugal force (rcf) for two minutes, separating the soluble from the insoluble parts of the mixture. The lysate was added to a 50mL falcon tube in which 10mL of binding buffer was mixed with 400µL of sodium acetate (pH 5.2, 3M). This mixture was then placed in a High Pure Extender Assembly (HPEA) tube and centrifuged at 1500 rcf for 8 minutes in a 50 mL Thermo Scientific TX-400 Swinging Bucket Rotor. Each HPEA's column was removed and inserted into a clean collection tube before centrifuging again at 18500 rcf for 2 minutes. To each column, 450µLof wash buffer from the high pure viral nucleic acid kit (HPVNAK) was added and the mixture was centrifuged for one minute at 8000 rcf. The columns were then placed into fresh collection tubes before another round of washing during which 450µL of wash buffer from the HPVNAK was added to each column followed by one minute centrifugation at 8000 rcf. Resulting columns with washed DNA were placed in 1.5 silicon tubes to which 50µL of TET was placed in the middle of the columns. The columns were incubated at room temperature for three minutes and centrifuged for one minute at 18500 rcf. Another round of adding 50µLof TET followed by incubation and centrifugation was performed and the final 100µL of DNA extract was stored at -20°C until further downstream use.

Extracts were thawed and shaken before 25µLfrom each extract was aliquoted into separate PCR strip tubes. Extracts were UDG-half treated by adding 25µL mastermix containing 0.5µL 20mg/ml BSA, 6µL 10x Buffer Tango, 6µL 10mM ATP, 3.6µL 1U USER enzyme, 0.2µL 25mM each dNTPs, and 8.7µL UV HPLC-water to each PCR strip tube. The resulting solutions were incubated for 30 minutes at 37°C and 10 minutes at 12°C. The UDG reactions were inhibited by adding 3.6µL 2U UGI to each PCR strip tube and incubating at 37°C for 30 minutes and again at 12°C for one minute. Blunt end repair was done by addition of 1.65µL 3U T4 DNA Polymerase and 3µL 10U T4 Polynucleotide Kinase, followed by incubation at 25°C for 20 minutes, and then at 12°C for 10 minutes. The resulting mixtures were purified with MinElute kits followed by elution in 20µL Elution buffer (EB) mixed with 0.05% tween. Ligation of Illumina adapters was performed through the mixture of 18µL eluate from the previous step with 1µL 5U Quick Ligase, 1µL 10µM Adapter Mix and 20µL of 2x Quick Ligase Buffer. The resulting solution was incubated for 20 minutes at 22°C and purified using a MinElute kit, followed by elution in 22µL EB containing 0.05% tween. Adapter fill in reactions were done by adding 20µL of eluate from previous step to 2µL 8U Bst 2.0 Polymerase, 0.2µL 25mM dNTPs, 4µL 10x Isothermal buffer, and 13.8µL UV HPLC-water and incubating at 37°C for 30 minutes followed by 80°C for 10 minutes. The resulting DNA libraries were stored at -20°C until indexing.

Library-specific and unique index combinations were ligated to both 5' and 3' ends of DNA fragments in each library via an indexing PCR. The total volume of each library was split into four different indexing PCR reactions which were done by mixing 2µL 10µM P5 index, 2µL 10µM P7 index, 1µL 2.5U Pfu Turbo Polymerase, 1µL 25mM each dNTPs, 1.5µL 20mg/ml BSA, 10µL 10x Pfu Turbo Buffer, 73.5µL UV HPLC-water, and 9µL of DNA library. The resulting mixture was amplified in a thermocycler with initial denaturation at 95°C for 2 minutes, followed by 10 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute, and finally 72°C for 10 minutes. The indexed libraries of the same sample were pooled and purified with a MinElute kit. Libraries were then quantified using qPCR and PCR amplified to contain 10^13 copies of DNA.

Resulting libraries were shotgun sequenced (~5,000,000 reads, either paired end with 50 cycles or single end with 75 cycles) to assess the library complexity and degree of human DNA preservation (% endogenous DNA, aDNA damage). Libraries with more than 0.1% endogenous DNA were deemed adequately preserved and selected for an in-solution hybridization enrichment (Fu et al. 2014) that targets ~10,445 kB on the NRY ("YMCA capture"). YMCA captured libraries were single end sequenced with 75 cycles to an average depth of 40 million reads per YMCA library. Libraries were not pooled prior to this capture method. After the enrichment the libraries were amplified to a concentration of 10 nM followed by a single end sequencing with 75 cycles to an average depth of 40 million reads per YMCA library.

All libraries were processed using EAGER (Peltzer et al. 2016), a modular tool that streamlines the processing of libraries from FastQC and quality filtering to mapping and duplicate removal. Sequencing adapters were clipped with AdapterRemover v2.2.0 (Schubert et al. 2016), and merged for paired-end sequencing with all reads of length <30bp discarded. The remaining reads were mapped to the human reference genome hs37d5 using BWA

v0.7.1 (Li et al. 2009) with a quality filter of q30. PCR duplicates were removed using dedup v0.12.2 (Peltzer et al. 2016).

Derivation of our method for estimating the pairwise time to most recent common ancestor (TMRCA)

We are interested in estimating the *total* amount of evolutionary time that has passed between two samples, denoted τ_{ij}, which can be separated into three non-overlapping intervals: the total evolutionary time until the last substitution occurs, and the total amount of evolutionary time that passed after the final substitution for samples *i* and *j* respectively, denoted

$$\tau_{ij} = t_{ij}^{*} + \delta_{ij}^{i} + \delta_{ij}^{j},$$

Respectively, where $t_{ij}^{*} = 2t_{ij}^{s} + t_{ij}$ (see Figure S1).

We begin by estimating the total evolutionary time between the ith and the jth samples, denoted t_{ij}^{*},up until the final substitution occurred. Let $N_{ij} > 0$, be the total number of overlapping SNPs., let $K_{ij} > 2$ be the number of observed segregating sites, and let $\lambda_0 > 0$ be the rate of substitutions per site per calendar year.

We assume that each substitution occurs according to a Poisson process with rate $\Lambda_{ij} = \lambda_0 N_{ij}$, relative to the number of overlapping SNPs for individuals i and j. Hence, for some evolutionary time $t > 0$, the total number of observed substitutions has an Erlang distribution with probability density function (pdf)

$$L(\, t \mid K_{ij}, \Lambda_{ij}) = \frac{\Lambda_{ij} t^{(K_{ij}-1)} e^{-\Lambda_{ij} t}}{(K_{ij}-1)!}.$$

Hence, if we assume that $K_{ij}$and $\Lambda_{ij}$are known, then we may look for the optimal value of t that maximizes the pdf. We do this by considering the log-likelihood function

$$l\big(\, t \mid K_{ij}, \Lambda_{ij}\,\big) = ln\left(\frac{\Lambda_{ij}}{(K_{ij}-1)!}\right) + \big(K_{ij}-1\big) ln(t) - \Lambda_{ij} t.$$

Hence, we have that

$$\frac{dl\big(\, t \mid K_{ij}, \Lambda_{ij}\,\big)}{dt} = \frac{K_{ij}-1}{t} - \Lambda_{ij}.$$

If we set the first derivative of the log-likelihood to zero, we obtain a candidate maximum likelihood estimate (MLE)

$$\widehat{t_{ij}^{*}} = -\frac{K_{ij}-1}{t^2},$$

and since $(K_{ij}-1), t^2 > 0$, it must be that $\widehat{t_{ij}^{*}}$ is a maximum likelihood estimate. We also use the property that the variance of a single, unknown parameter is approximately the negative of the reciprocal of the Fisher information, e.g.

$$\hat{\sigma}^2 = \frac{K_{ij}-1}{\Lambda_{ij}^2}.$$

It must also be considered that the final substitutions probably did not occur at time t_i and t_j, and that some time will have passed with no substitution events since the last substitution. Hence, to our MLE for the time until the final substitution $\widehat{t_{ij}^{*}}$, we must add some additional amount of time.

To achieve this we make the standard assumption that the time until the next substitution *would* have occurred for sample $k \in \{i,j\}$ is exponentially distributed, that is, $X_{ij}^{k} \sim Exp(\Lambda_{ij})$. We also assume that we observed a random proportion of the interval until the next substitution, denoted $U_{ij}^{k} \sim U(0,1)$. Finally, we denote the amount of time that has passed in the final interval $\delta_{ij}^{k} = U_{ij}^{k} X_{ij}^{k}$, where U_{ij}^{k} and X_{ij}^{k} are statistically independent. Note then that $E[\delta_{ij}^{k}] = \frac{1}{2\Lambda_{ij}}$ and $Var(\delta_{ij}^{k}) = \frac{5/12}{\Lambda_{ij}^2}$.

Now that we have derived estimates for t_{ij}^{*} and the δ_{ij}^{k}, we transform these to find an estimate of the TMRCA of samples i and j, relative to the present day. Note that the true total amount of shared evolutionary time between the final substitution between samples i and j can be rewritten in terms of the shared and unshared branch times (as in Figure ??)

$$t_{ij}^{*} = 2t_{ij}^{s} + t_{ij}$$

where

$$t_{ij} = (t_j + \delta_{ij}^{j}) - (t_i + \delta_{ij}^{i}).$$

Since $E[\delta_{ij}^{k}] = 1/2$, it can be can be shown that

$$E[t_{ij}] = t_j - t_i \text{ and } Var(t_{ij}) = \frac{1/6}{\Lambda_{ij}^2}.$$

Note that since

$$t_{ij}^{*} = 2t_{ij}^{s} + t_{ij} \Rightarrow t_{ij}^{s} = \frac{1}{2}(t_{ij}^{*} - t_{ij})$$

a natural choice of estimator for the length of shared evolutionary time for individuals i and j would be

$$\widehat{t_{ij}^{s}} = \frac{1}{2}\left(\widehat{t_{ij}^{*}} - \widehat{t_{ij}}\right)$$

yielding an estimator for the TMRCA for individuals i and j, relative to the present day would be

$$\hat{T}_{ij} = t_i + \delta_{ij}^{i} + \hat{t}_{ij} + \widehat{t_{ij}^{s}},$$

for which a best estimator can be simplified to give

$$\hat{T}_{ij} = \frac{1}{2}\left(t_i + t_j + \frac{K_{ij}}{2\lambda_0 N_{ij}}\right).$$

Finally, we have that

$$\begin{aligned}
Var\left(\hat{T}_{ij}\right) &= Var\left(t_i + \delta_{ij}^{i} + \hat{t}_{ij} + \widehat{t_{ij}^{s}}\right) \\
&= Var\left(\delta_{ij}^{i} + \tfrac{1}{2}\left[t_j + \delta_{ij}^{j} - t_i - \delta_{ij}^{i}\right] + t_{ij}^{*}\right) \\
&= Var\left(\frac{1}{2}\delta_{ij}^{1} + \frac{1}{2}\delta_{ij}^{j} + \frac{1}{2}t_{ij}^{*}\right) \\
&= \frac{1}{4}Var\left(\delta_{ij}^{1} + \delta_{ij}^{j} + t_{ij}^{*}\right) \\
&= \frac{1}{4}Var\left(\frac{5/12}{\Lambda_{ij}^{2}} + \frac{5/12}{\Lambda_{ij}^{2}} + \frac{K_{ij}-1}{\Lambda_{ij}^{2}}\right) \\
&= \frac{K_{ij}-1/6}{\Lambda_{ij}^{2}}.
\end{aligned}$$

To test the performance of our method, we simulated 10,000 realisations of the following process. We used a grid search for the number of overlapping SNPs ranging from 20,000 to 10,000,000, (the observed values from our filtered data set) and two randomly sampled branch lengths from a log-Normal distribution from between 20,000 and 175,000 years to sample a random tree and number of overlapping sites. We used the simSeq function from the phangorn package to produce a pair of sequences (using a Jukes-Cantor model of substitution and a substitution rate of 4.5×10^{-10}substitutions per site per year per individual), from which we could count the number of pairwise segregating sites (Schliep 2011).

We found that 94.69% of the known simulated TMRCAs were within the 95% confidence intervals as calculated by our method. We found that the accuracy of our method was uncorrelated with the number of overlapping sites (p=0.951), the true TMRCA (p=0.961), or a combination of both (p=0.193) indicating that our method is unbiased for both the depth of time, and the number of overlapping sites observed in our data.

TMRCA Estimation

To count the number of pairwise-segregating sites between two individuals, we began by making a fasta file of aligned consensus sequences for all each bam file, and performing the following quality filter; for each sequence we considered only sites for which we had at least three reads, with a minimum allele frequency less than 10%, and called the majority allele (as suggested by Petr *et. al*. 2020) We then took the aligned consensus sequences, and calculated the number of overlapping sites for which the pair had both recorded a consensus call, and the number of pairwise segregating sites for each pair. For the substitution rate calibration we kept only pairs with >3,000,000 overlapping sites, and for the within-H2 estimates we kept only pairs with 200,000 overlapping sites, >1 segregating sites (as required by the method).

We calibrated our (relative NRY) substitution rate by fixing the mean estimated TMRCA of all Y-haplogroups A0 and all other Y haplogroups at 161,300 ybp [https://www.yfull.com]. To test if there was any effect from DNA damage or sequencing error, we first calculated a substitution rate for TMRCA estimates based on modern/modern pairs, and modern/ancient pairs. Our separate substitution estimates were within 0.687% of each other, indicating no significant increase in the substitution rate due to using ancient samples. When we estimated the substitution rate using the combined data, we found a substitution rate of 4.5×10^{-10}, which falls within the confidence intervals of existing estimates (Xue et al. 2009; Mendes et al. 2013).

Supplementary Information

Supplementary tables are too big to fit into thesis and have been burnt onto CD which accompany this thesis.

Table S1: Sample Metadata
Table S2: New Library Metadata
Table S3: Leubingen Performance
Table S4: Coverage on YMCA without YCC
Table S5: SNP Detection H2
Table S6: SNP Detection H2d
Table S7: SNP Detection H2m

Supplementary Figures

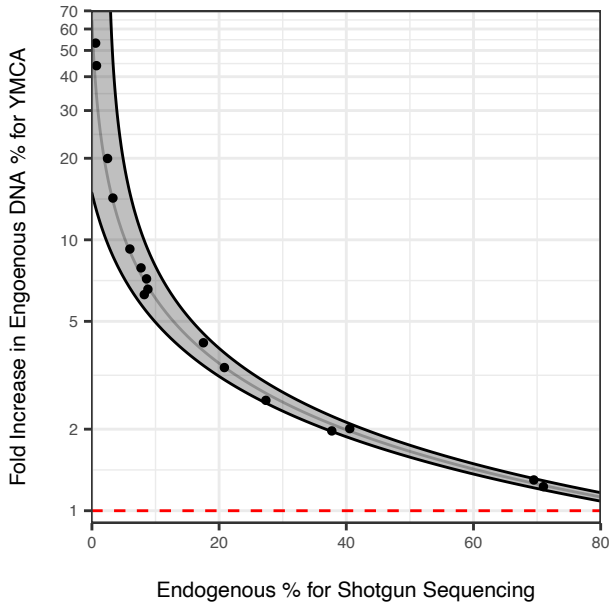

Figure S1: *Fold-increase in endogenous human DNA % (y-axis) for YMCA compared to shotgun sequencing (x-axis). The shaded region indicates a 95% prediction interval, and the red dashed line indicates no improvement (a fold-increase of one).*

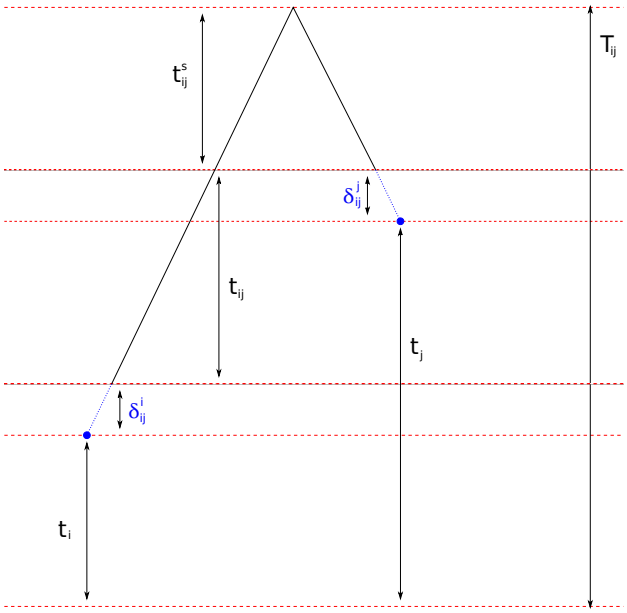

Figure S2: *Relative branch lengths: T_{ij} is the time to most recent common ancestor (T_{MRCA}) for samples i and j relative to the present day, t_i and t_j are the calibrated ages of the samples relative to the present day, where $t_i < t_j$, $t_{ij}$is the additional evolutionary time since the most recent common ancestor (MRCA) for sample i (compared to sample j), t_{ij}^{s} is the shared evolutionary time per sample since the MRCA, and the δ_{ij}^{k} are the times between the final mutation for each lineage, and the sampling date.*

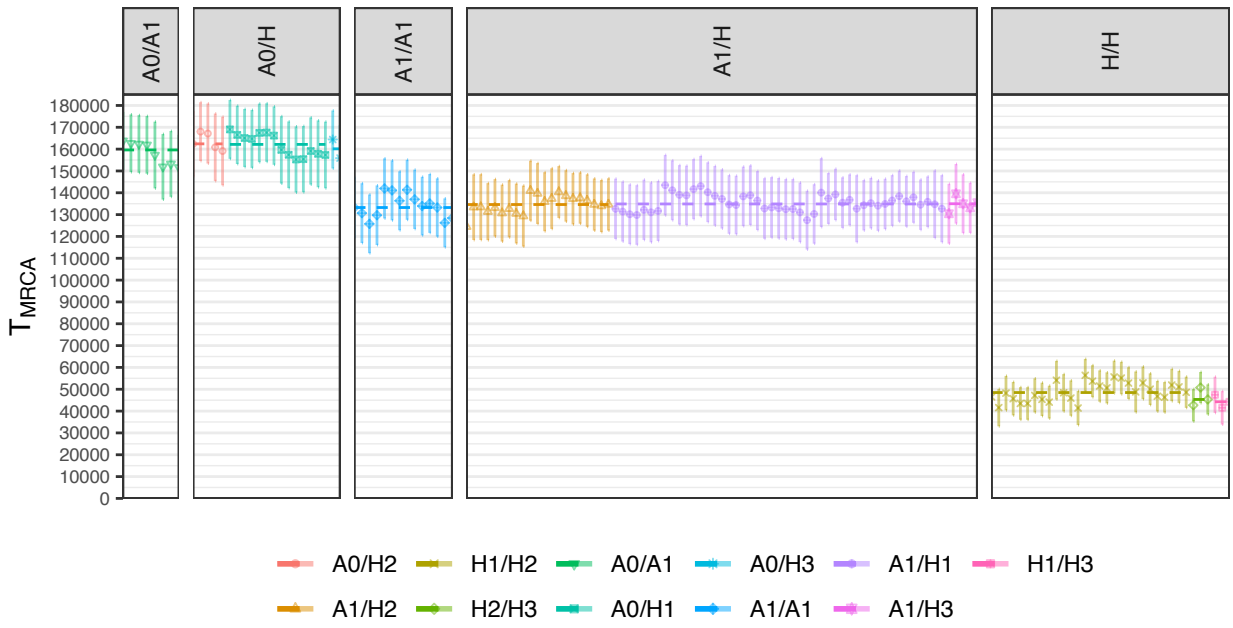

Figure S3: *Estimated T_{MRCA} (y-axis) for each H2 sample with pre-published individuals from Y haplogroups A0, A1, H1 and H3 (facets), calibrated by the split time of ~163 kya of A0 with all other Y haplogroups. The dashed line indicates the mean estimate, and error bars indicate 95% confidence intervals for individual observations.*

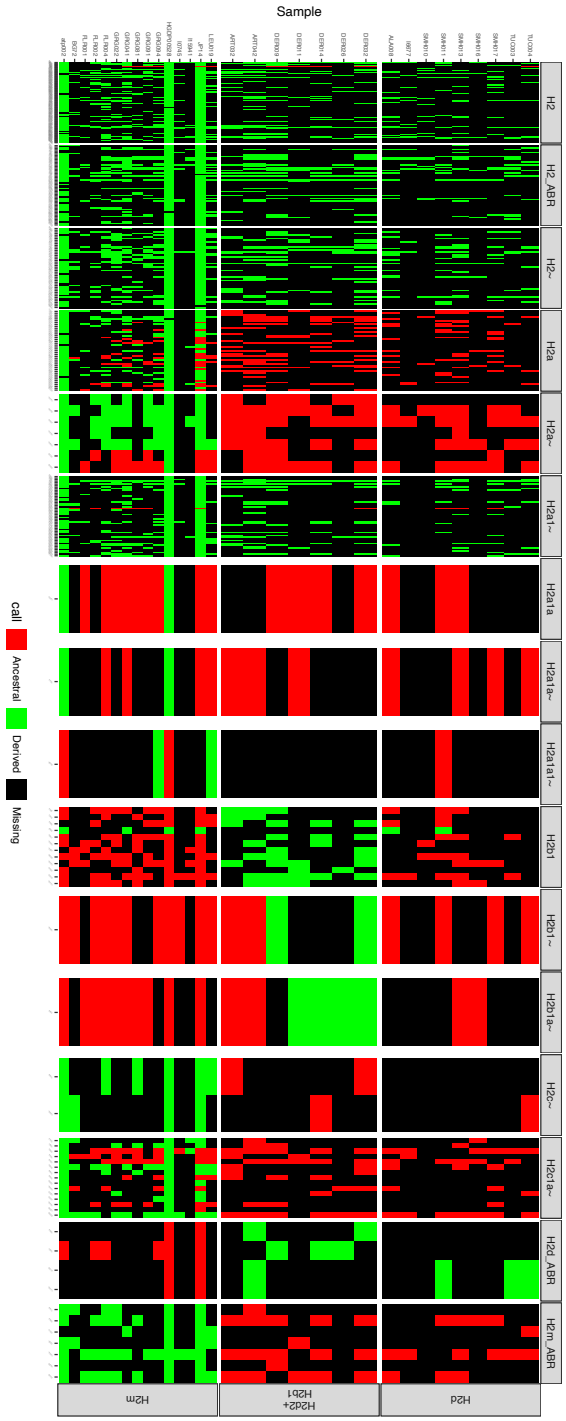

Figure S4: *Presence and absence of SNPs for our H2 sub-haplogroups (note that we include our potential further sub-haplogroups H2m1, H2m2, H2d1 and H2d2) with samples on the y-axis, and SNP positions on the x-axis. Columns facets represent ISOGG SNP branch assignments, and potentially newly identified SNPs (denoted with "ABR"), row facets indicate our H2 sub-haplogroups assignments. Green, red and black indicate derived, ancestral or missing forms of SNPs.*

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8. Discussion

The last two decades of archaeogenetic research have made it clear that large-scale migrations played an important role in shaping the distribution of ancient cultural and biological diversity in Europe (Haak et al., 2015; Allentoft et al., 2015; Olalde et al., 2018). However, open questions remain concerning the frequency of large-scale migrations, their geographic extent, their cause, their nature, and their impact on local societies and communities. The four studies (manuscript A, B, C, and D) presented in this thesis allow for new insights into these questions to be gained, thereby extending our understanding of the prehistory of Europe.

Although Europe is the best studied continent from an archaeological and archaeogenetic perspective, most previous genetic studies have employed low density sampling strategies, and, as a result, revealed predominantly the major demographic changes which involved the appearance of new and never before seen genetic lineages (e.g. the spread of "farmer" and "steppe" ancestry). However, it is clear from the archaeological record that human prehistory has been more complex than the initial broad-brush patterns emerging from the first genomic studies. Current efforts, including chapters in this thesis, aim to fill in spatial and temporal gaps, allowing for more nuanced models of the human past. Here, the focus on Bohemia (manuscript A and B), a region which has attracted many different cultural groups to its fertile lands, has allowed for subtler genetic shifts, social processes, and social behaviours to be deciphered (manuscripts A, B, C, and D).

New insights into the Neolithic transition in Europe

The genetic makeup of Neolithic individuals from Bohemia (manuscript A) falls in the diversity expected from previous studies on Neolithic Europe and is in stark contrast to the ancestry profile of autochthonous hunter-gatherers. This finding is in accordance with the large population turnover associated with the arrival of agriculture and animal husbandry in Europe.

This large genetic turnover during the Meso-Neolithic transition is also mirrored in the turnover in HBV genetic diversity (manuscript C), whereby HBV strains common in European hunter-gatherers are replaced by a West-Eurasian Neolithic-to-Bronze-Age (WENBA) lineage, which appears for the first time and is the only lineage found among Neolithic Europeans (5,500-3,000 BCE). Interestingly, this WENBA HBV lineage spreads as far east as the Moscow and Samara regions in this time period, even though farming (as found in most of central and western Europe) is not attested in these regions. Since HBV is transmitted through contact with bodily fluids (e.g. blood, semen), it is likely that farming groups who brought this lineage to Europe had physical contacts with hunter-gatherers to whom they spread this HBV strain, even though considerable farmer ancestry is not found in hunter-gatherers of eastern Europe, and is first attested in this region in individuals of the Yamnaya cultural complex ~3,000 BCE (Wang et al., 2019). It remains unclear precisely where this WENBA transmission from farmers to hunter-gatherers may have taken place.

In addition to the evidence of horizontal exchange and contacts between Neolithic farmers and hunter-gatherers, added insights into the spread of agriculture in Europe is obtained

through increased resolution in Neolithic Y chromosomes (manuscript D). It has been hypothesised that agriculture spread to Europe via two routes, an inland route along the Danube reaching central Europe, and another along the northern coastline of the Mediterranean Sea into Italy, Iberia and France. By developing a capture array targeting mappable regions of the Y chromosome, the phylogenetic tree of haplogroup H2 was resolved and a phylogeographic signal was uncovered, with central European (from Germany and Czech Republic, H2d for "Danubian") forming a distinct clade to southern and western European (from Italy, Spain, and France, H2m for "Mediterranean") H2 lineages.

Previously unidentified genetic turnovers within Neolithic and Early Bronze Age Europe

The genetic origins of Funnelbeaker-Baalberge individuals in Bohemia

In addition to the previously attested major population turnovers in central Europe associated with the spread of agriculture ~5,500 BCE and "steppe"-related ancestry ~3,000 BCE, the densely sampled data set from Bohemia reveals at least another four, previously unaccounted for, demographic shifts in central Europe (manuscript A). The first of these is the genetic shift associated with the appearance of the Funnelbeaker-Baalberge (~3,900-3,600 BCE) culture in Bohemia. Funnelbeaker-Baalberge individuals from Bohemia share significantly more ancestry with Funnelbeaker-Baalberge individuals from Saxony-Anhalt (Germany) than with preceding Jordanów individuals from Bohemia, implying a large-scale (significantly greater than 50%) population turnover accompanying the spread of the Funnelbeaker-Baalberge culture to Bohemia. It is unclear, however, from where this demographic change originated. The current data are compatible with an origin in today's Germany, followed by spread to Bohemia and other regions. Or, an alternative scenario involving a different origin followed by spread to both Germany and Bohemia is also compatible with the data. Based on the Loschbour-like hunter-gatherer ancestry found in the Funnelbeaker-Baalberge individuals, its origin is likely to be along the northern European plain, where similar hunter-gatherer ancestry has been found (Lipson et al., 2017; Fernandes et al., 2018; Rivollat et al., 2020). Denser sampling of Funnelbeaker along with pre-Funnelbeaker associated individuals will shed light on where this culture is autochthonous and its pattern of spread.

The genetic origins of Globular Amphora individuals in Bohemia

The second genetic turnover in Bohemia involved individuals of the Globular Amphora culture (GAC), who are genetically more similar to previously published GAC individuals from Poland and Ukraine, thus likely representing migrants from the north/northeast (manuscript A). The GAC appears in Bohemia around 3,000 BCE and only exists there for 200-300 years. The demic nature of the spread of the GAC to Bohemia may offer important insights into the contemporaneous "steppe"-related migrations. The arrival of GAC individuals to Bohemia suggests that Corded Ware associated individuals may not have been the only cultural group on the move at this time. It is not clear whether the movement of GAC individuals was in response to the expansion of Corded Ware (CW) individuals, or if both were moving in response to a different stimulus. Nevertheless, the GAC and CW arrival in Bohemia around (or shortly after) 3000 BCE, along with the pre-existing Řivnáč cultural

group, resulted in Bohemia being populated by three culturally and genetically distinct groups. These three groups likely also interacted and exchanged members of their societies. For example, two individuals buried in early CW archaeological contexts and one buried in a Řivnáč archaeological context show genetic profiles resembling those of GAC individuals. Unfortunately, sample sizes of each group in Bohemia are still too small to confidently infer rates of cross-cultural biological exchange, but scenarios of complete isolation seem unlikely.

The genetic shift between early and late CW individuals in Bohemia

The third newly identified genetic shift in this thesis is the shift between early and late CW groups. Due to the increased resolution, both in sampling density and chronology (i.e. across the entire CW temporal range), manuscript A is able to divide major cultural groups of the European 3rd millennium BCE into early and late phases and study how their gene pool changed through time. In doing so, we find that the CW gene pool undergoes significant changes throughout this cultural group's existence in Bohemia. The most obvious change is that of the frequency and diversity of Y chromosome haplogroups. Early CW (2900-2600 BCE) males in Bohemia carry five different Y chromosome haplogroups (R1b-U106, R1b-L151, Q1, I2, R1a-M417(xZ645)), with R1b-L151 being the most frequent at 45% (5/11) and R1a-M417(xZ645) being the second most common at 27% (3/11). In contrast, late CW males in Bohemia are found to carry only two Y chromosome lineages, one of which (R1a-M417(xZ645)) is almost found in every male in the dataset (10/11, 91%). This drastic change in Y chromosome diversity (from five, relatively evenly distributed lineages to a single, dominant lineage) was shown to be unlikely to be explained by random drift and was complemented by autosomal analyses which also suggested non-local influences between early and late CW in Bohemia. Such a finding underscores the importance of filling sampling gaps in the archaeogenetic record and attests to the speed at which genetic diversity can change (over 100s of years), even within individuals associated to the same archaeological material culture. From the perspective of geography, Bohemia's central location on important rivers (e.g. Elbe) along with its attractive fertile lands means it was it was likely never isolated from neighbouring regions and demographic influences. In this context, finding a changing genetic landscape, even within indivdiuals associated to the same archaeological culture, may not be so surprising.

The origin of Únětice individuals in Bohemia

The fourth genetic shift identified for the first time in manuscript A coincides with the origin of the Early Bronze Age Únětice culture in Bohemia. Although similarities in pottery between the Bell Beaker (BB) "Begleitkeramik" and aspects of Únětice cups have been previously interpreted by archaeologists as evidence for a large degree of population continuity between these two groups, we find this cultural change was associated with a ≥40% autosomal and ≥80% Y-chromosomal turnover broadly originating from the northeast. After the large Y chromosome shifts seen in early CW, late CW, and BB males, this represents the fourth large Y chromosome shift in Bohemia within the space of ~800 years, and underscores the large degree and speed of genetic change in 3rd millennium BCE Bohemia, and likely central Europe in general.

Population turnovers involving genetically less distinct individuals

Previous studies have inferred large-scale migrations/expansions only when new ancestries arrived in certain regions of Europe. For example, the "Anatolian Neolithic" ancestry which for the first time appeared in central Europe with early farming populations, the "steppe"-related ancestry which first appeared in CW individuals, along with "steppe" ancestry's arrival in Iberia and the Atlantic archipelago. When a new, never before sampled, ancestry arrives in a region for the first time, it is clear that the movement of people, whether it be through migration or range expansion, is the likely process by which this new ancestry is introduced.

In contrast, finding subtler genetic changes across cultural transitions does not necessarily imply a scenario of population continuity without non-local contribution. However, cases of subtler genetic shifts require more data and denser sampling from nearby regions to directly test whether certain archaeological cultures spread through demic or cultural diffusion. For example, the genetic turnover detected between Jordanów and Funnelbeaker-Baalberge individuals in Bohemia (manuscript A) required a large enough sample size to be able to accurately infer and compare allele frequencies in both Bohemian Jordanów (n=5) and Bohemian Funnelbeaker (n=30) indivdiuals, as well as non-Bohemian Funnelbeaker (Saxony-Anhalt, n=6) individuals as a reference population to directly test whether Bohemian Funnelbeaker are genetically closer to local Jordanów or non-local Funnelbeaker individuals (from Saxony-Anhalt, Germany). In doing so, the results showed a significantly closer genetic relationship between Bohemian and Germany Funnelbeaker individuals compared to Bohemian Funnelbeaker and Bohemian Jordanów individuals, a pattern consistent with a large population turnover with the arrival of the Funnelbeaker culture in Bohemia. Since Neolithic groups in central Europe (e.g. Jordanów and Funnelbeaker) are genetically similar (i.e. both carry ~80% Anatolian Neolithic-like ancestry), larger sample sizes are required to identify genetic differences. Without the increased sample sizes and sampling densities presented in manuscript A, the identified genetic shifts mentioned previously would have gone unnoticed, as they had until now.

Increasing resolution

In addition to increasing sampling density, increased resolution can also be achieved by employing more complex methods than the SNP-based methods used in this thesis. SNP-based methods (e.g. PCA, f-statistics, qpWave/qpAdm, Fst) allow for general, genome-wide similarities to be quantified by comparing allele frequencies at many positions across the genome. It is unlikely that such SNP-based methods are currently utilising all information contained in the ancient genetic data generated.

Two attempts at extracting more resolution from ancient genetic data have been to utilise rare genetic variation and haplotypes. Since rare genetic variation is likely to have occurred recently in time, sharing patterns in rare genetic variation may be useful in revealing more recent demographic events (Schiffels et al., 2016; Amorim et al., 2018; Flegontov et al., 2019). Haplotype-based methods offer higher resolution by utilising patterns of co-inherited combinations of multiple genetic markers on the same chromosome. The coinheritance of these markers along DNA segments is largely dependent on the length of the co-inherited

segment as well as the rate of recombination along the segment. Since many different combinations of markers can exist along a chromosome, the finding of an identical haplotype shared between individuals can reveal a direct genealogical link. As recombination breaks down haplotype lengths over time, the length of the shared haplotype found in two individuals can also inform on the time depth (or number of generations ago) of the shared genealogical ancestor.

Although haplotype-based methods have been previously used in ancient DNA research (Martiniano et al., 2016; Martiniano et al., 2017; Cassidy et al., 2020), the low coverage, high error rate, risk of contamination and potential biases in capture data have posed a challenge to reliably imputing and phasing ancient genomes. However, current and future developments (Hui et al., 2020; Rubinacci et al., 2020; Ringbauer et al., 2020) in this sphere show promise in extracting even more insights into the structure and distribution of diversity within low coverage ancient genomes, and thereby elucidating further on ancient population structure, biological kinship, and even past effective population size (Fernandes et al., 2020). While rare genetic variation and haplotype-based methods hold promise, the approach of densely sampling regions (manuscripts A and B) and time periods also allows for finer population structure to be unravelled, and in doing so reveals aspects of past social processes.

Overall, the findings from manuscript A underscore the importance of achieving high sampling densities, not only to better understand a particular region of interest, but also to identify potential sources for demographic changes in nearby regions, as well as understanding the geographic extent of certain transitions. Increased cohort sizes of cultural/genetic units or entities are key to robust demographic inferences. For example, the source of the large non-local contribution from the northeast at the transition from BB to Únětice in Bohemia is of great interest but remains unidentified due to the low sampling density from Poland, Belarus and Baltic 3rd millennium BCE. In addition, the lack of high sampling density of ~2200-2000 BCE central Europe means that not much is known about the geographic extent of these genetic turnovers identified in Bohemia, for example with respect to the EBA groups in southern Germany (Straubing/Singen/Adlerberg) or the northern Bronze Age in general. At cross-regional scale, we do not currently know whether the north-eastern influence in Bohemian Únětice also other affected other Únětice groups in today's Poland, Germany and Austria, and if so, to what extent. As a result, additional data from these regions is needed to further detail the geographic expanse of these genetic transitions identified in Bohemia.

These results challenge our current understanding that large demographic changes occurred only with the advent of agriculture and arrival of "steppe"-related ancestry in Europe. Although these two processes introduced important ancestries to Europe, it is likely that other, yet to be identified, large-scale processes also helped shaped European genetic diversity over the last 8,000 years. This is also hinted at in manuscript C, for example, where a complete turnover in HBV lineages is detected at the advent of the Iron Age (~1,000 BCE), with the previously dominant WENBA lineage disappearing from the archaeogenetic record.

Insights into social processes in prehistoric Bohemia

The increased sampling densities in manuscripts A, B and C also provide new insights into the social processes and spheres of interaction in Neolithic and Early Bronze Age Bohemia.

Interactions between CW and pre-CW individuals

The first of these concerns the arrival of "steppe"-related ancestry with CW individuals in the early 3rd millennium BCE. Manuscript A reports the first individuals buried in early CW cultural contexts who lack "steppe"-related ancestry, and provides direct evidence of the interaction between, and incorporation of, pre-CW individuals into early CW society. Based on one interpretations of the ancient genetic data (Goldberg et al., 2017), the incorporation of pre-CW people into early CW society was hypothesised to have been a process driven by pre-CW (Neolithic) women being incorporated into early CW society (Kristiansen et al., 2017). This process may have been related to kóryos, a hypothetical Indo-European ritual or rite of passage into adulthood for young men, who – it is thought – would raid neighbouring settlements, gaining new territory and possibly access to mating partners (Anthony & Ringe 2015).

The new genetic data reveals that in each case (4/4) a person with a pre-CW genetic profile was buried in a CW context, the person is female, offering support to the notion of a sex-biased interaction between early CW and pre-CW people. Interestingly, no archaeological differences could be identified in graves containing early CW individuals with a high proportion of "steppe"-related ancestry and graves of individuals without "steppe" ancestry. Since graves are constructed by other members of the community, they often reflect how the community sees the individual. Consequently, the lack of archaeological differences between early CW graves of individuals with and without "steppe" ancestry likely reflects a high degree of integration and/or assimilation of pre-CW females into early CW society. It has also been hypothesised that these assimilated females may have brought in important knowledge about pottery making and some evidence of continuity of pre-CW and CW pottery exists (Furholt 2008; Beckerman 2017; Kolář 2018).

When looking into the genetic origin of the pre-CW females buried in an early CW context, we find them to be genetically diverse (manuscript A). Two of them appear to genetically resemble GAC individuals. However, when directly comparing whether these two are genetically closer to Bohemian or Polish GAC, they are not closer to Bohemian GAC, meaning a Polish GAC origin for these two females cannot be ruled out. The other two fall outside of the genetic variation commonly seen in pre-CW central Europe and each of the two with clearly different affinities, suggesting that the integrated females have diverse genetic origins.

The geographic distribution of the incorporated pre-CW females into early CW society suggests this process to have occurred across a wider geographic region (manuscript A). Two sites, Stadice in north-western Bohemia and Vliněves in central Bohemia, show evidence of pre-CW females buried in early CW cultural contexts. From Vliněves, three of the fifteen sampled (20%) early CW individuals lack "steppe"-related ancestry and from

Stadice one of the sampled three (33%). When considering that it is unlikely that all of the early CW individuals sampled in manuscript A come from the earliest CW horizon (~2900-2800) and that some may be from closer to 2600 BCE, the data suggests that finding early CW individuals without "steppe"-related ancestry may not have been uncommon and that several early CW communities across Bohemia comprised of individuals with vastly different genetic backgrounds, and possibly mother tongues and worldviews.

Under the scenario of migrating males in search of new territory, a reasonable motive for incorporating pre-CW females into their society may have been, at least in part, for procreation. Under such a scenario we may expect only females of reproductive age to have been incorporated into early CW society. Although three of the four incorporated females are adults (aged 30+), one of the females (VLI079) is a juvenile 5-7 years of age. It is not known whether she was assimilated together with her mother (who has not been sampled), however, other scenarios such as abduction or adoption as part of a trade, or offering in exchange for peace cannot be ruled out.

Although co-existing for ~300 years in time (2900-2600 BCE) and space (north-eastern Europe, e.g. Poland), GAC and CW individuals have been shown to have vastly different genetic affinities. GAC individuals were largely descendent of previous agricultural societies of Europe (e.g. Funnelbeaker) (Mathieson et al., 2018), whereas CW individuals shared much (~75%) of their ancestry with Yamnaya individuals of the Pontic-Caspian steppe, a genetic profile unlike anything seen in Europe prior to 3,000 BCE (Haak et al., 2015; Allentoft et al., 2015). This large genetic difference between GAC and CW implied a long biological isolation between the ancestors of these two groups. In addition, their significantly different burial rituals (e.g. relatively strict gender specific body positions and associated grave goods) likely also suggest a fundamentally different ideology and/or worldview.

In light of their co-existence but large genetic and possibly ideological differences, it is interesting to try to gain insights into the modalities of interaction and biological exchange. Approximately equal numbers of early CW and GAC individuals have now been studied from an archaeogenetic perspective (n=~44). Among the GAC individuals studied, not a single one shows evidence of carrying "steppe"-related ancestry. In contrast, four early CW individuals have now been found to carry no "steppe"-related ancestry, with two of those having GAC-like genetic profiles. This pattern of exchange suggests early CW societies were more open to incorporating foreigners than GAC societies. This may have been related to early CW migrants expanding into new territories and having more to gain from locals who may have had important knowledge needed to survive or thrive in central Europe.

In contrast to early CW individuals without "steppe"-related ancestry, focussing on early CW individuals with the highest amount of "steppe" ancestry may reveal insights into the sex ratio of the earliest migrating individuals. Previous genetic (Goldberg et al., 2017; Mittnik et al., 2019) and archaeological (Hübner 2005; Kristiansen et al., 2017) research has suggested the earliest CW migrants to have been predominantly males, with estimates of approximately ten times more migrating males than females (Goldberg et al., 2017). The early CW data presented in manuscript A suggests that females were well represented among early migrating individuals from the east, with the individual with the highest amount of "steppe" ancestry being female and three females among the five with highest

amount of “steppe” ancestry. However, these data do not rule out a sex bias in the earliest migrating groups from the east since the females with the most amount of “steppe” ancestry in the Bohemian dataset may not have been among the earliest migrating groups, but may have been incorporated into early CW society from nearby Yamnaya groups, e.g. Yamnaya in Hungary.

Social insights through distribution of Y chromosome diversity

Worth noting and informative is also the distribution of Y chromosome diversity in early CW. From published Yamnaya, CW and BB data, it appears as though all three groups had limited Y chromosome diversity, each with a single, dominant Y chromosome lineage (R1b-Z2103 in Yamnaya, R1a in CW, and R1b-P312 in BB) present in >70% of males. However, early CW Y chromosome diversity is comparatively high, with 5 different Y chromosome lineages and the most common lineage present in only 45% of males (R1b-L151). It is not clear whether this pattern represents aspects of the early migrating groups. It is possible that early migrating CW groups were made up of diverse male clans or that they had a different social organisation to other, more established groups. We find that the Y chromosome diversity in early CW is reduced to a single, dominant lineage in Bohemian late CW (91% R1a-M417(xZ645)). This process may reflect the establishment of a new social structure in which some males (possibly men in power) had a higher chance of producing offspring, although the data does not exclude a scenario in which new R1a-M417(xZ645) lineages were introduced from outside of Bohemia amid the same social organisation.

This (sudden) dominance of a single Y chromosome lineage is then taken a step further in the BB period where every male (n=33) carries the same Y chromosome lineage. This new lineage R1b-P312 has never before been sampled in Bohemia, implying a complete turnover in Y chromosome lineages between CW and BB periods (~2,400 BCE). Surprisingly, CW and BB were also partially contemporaneous in Bohemia for at least 100 years and sometimes also found at the same site (e.g. Vliněves). However, despite this temporal and geographic proximity, no Y chromosome sharing is found between CW and BB males, and not a single Y chromosome lineage from pre-BB times made it into BB males. Such a pattern can suggest two things. Firstly, a new and strict social structure in BB society, something akin to a “brotherhood” in which all males descend from the same man within the recent past. Secondly, a type of isolated mating network between CW and BB males in which males born into either cultural context were buried almost exclusively in the cultural context of their birth, and were rarely (or perhaps never) given the other burial.

Towards the end of the 3rd millennium BCE, the social organisation characteristic of the BB societies seems to have broken down amid the ≥40% contribution from the northeast at the beginning of the Únětice period in Bohemia. This is evident from the Y chromosome diversity in pre-classical Únětice (2,200-2,000 BCE). Despite having sampled only 20 males in pre-classical Únětice (compared to 33 in BB), they carry 5 different Y chromosome lineages with the most frequent lineage being present at 40%. This change in social organisation may be related to the change from the relatively strict gender-based body orientation of CW and BB to the burial customs of Únětice, in which both sexes were laid to rest in similar orientations.

Zooming into the social organisation and behaviour within an Early Bronze Age community

Recent advances in enrichment techniques (Maricic et al., 2010; Mathieson et al., 2015) and understanding of skeletal elements which preserve ancient DNA well (Pinhasi et al., 2015; Parker et al., 2020) have increased our ability to obtain authentic ancient DNA, making detailed studies of whole cemeteries feasible. Such studies hold the potential to reveal kinship structure, social organisation and, when combined with other data, the lifeways of ancient individuals and communities.

Manuscript B's detailed investigation of an Early Bronze Age Únětice cemetery in Mikulovice (Ernée et al., 2020), eastern Bohemia, also sheds light on this community's social organisation and behaviour. Here, genetic, and anthropological data (age at death) is used to reconstruct pedigrees of biological kinship in the light of the archaeological context (orientation, grave goods, radiocarbon dates, etc.) which, in conjunction with stable isotope (Strontium and Oxygen) data, reveal aspects of the community's social organisation.

This community likely practised a higher degree of patrilocality than matrilocality, a finding consistent with other studies of nearby contemporaneous groups (Knipper et al., 2017; Mittnik et al., 2019). Interestingly, despite being in a period of clear continental trade with evidence of a high degree of exotic (imported) grave goods, both isotope and genetic analyses revealed a low incidence of outliers (non-locals), suggesting the community was predominantly made up of locals or incomers from nearby. As a result, the acquisition of exotic artefacts may have been mediated through a limited subset of the community who was mobile.

Social behaviour appears to have been diverse at Mikulovice with evidence of both long-term relationships between a man and woman (e.g. a couple having six children together with no evidence of children to other partners) and individuals having children with different partners. However, despite the high incidence of locals, no evidence of inbreeding between closely related individuals is found. Both men (n=4) and women (n=2) were found to have children with different partners, including a case of a woman having offspring with two brothers, which might indicate duties of provision (or retention of status quo) regulated through familial ties. The high incidence of missing individuals in the reconstructed pedigrees likely precludes a detailed understanding of the mating behaviour at Mikulovice, and suggests it was part of a larger community where some of the missing individuals may have been buried.

Surprisingly, some isotope outliers were found to be biologically very well integrated into the Mikulovice community. For example, three Oxygen outlier individuals who may have spent a significant portion of their childhood in the northeast (possibly Baltics) had parents and/or grandparents also buried at Mikulovice. This suggests a more complex pattern of mobility than may have been previously appreciated, with some individuals perhaps being born in Mikulovice, spending time away from Mikulovice, before returning and being buried there. Importantly and more generally, the finding of isotope outliers who appear well integrated into their community implies that not all isotope outliers may have been complete foreigners to where they were buried. Perhaps a network of distant communities existed in which people would spend portions of their life where they may have sought

education, training, experience or spiritual enlightenment, which they eventually brought back to their community of birth or origin. It is through such mobility that exotic items, which are commonly found as grave goods at Mikulovice, may have been brought in.

Future directions

Results from the four manuscripts presented in this thesis have shown that although much has been learnt about Europe's prehistory in recent years, more is awaiting to be discovered and understood. Currently, at least in central and eastern Europe, many sampling gaps remain, and since Eastern Europe played an important role in being the source of major migrations and/or expansions, which shaped much of the continent's biological and cultural diversity, the sampling gaps in these regions are likely to hold important insights into these processes.

The most critical geographic sampling gap which remains in the archaeogenetic record is that of north-eastern Europe. Our knowledge of the ancient genetic diversity in regions such as Belarus, Poland, Ukraine and western Russia remain poor. Manuscripts A and B have shown that central Europe underwent several periods of influences likely originating from the northeast (e.g. GAC, early CW, Únětice), emphasising the importance of these regions in understanding the origin of several major genetic transitions in the prehistory of Europe. The closing of these sampling gaps would not only elucidate the genetic histories of these regions, but also provide important genetic data from potential source populations which expanded and transformed other regions of Europe.

In addition to obtaining genetic data from hitherto poorly sampled regions and time periods, the results presented in this thesis could be complemented by other scientific analyses. For example, stable isotope analyses of the early CW individuals will shed light into their geographic origin and diet. Especially interesting would be to compare the stable isotope signatures of males and females, including those lacking "steppe"-related ancestry. Information on diet and inheritance of grave goods in Mikulovice will shed further light on aspects of behaviour and social organisation.

Recent developments in haplotype-based methods have shown promise in revealing aspects of population history not previously possible. These include the incidence of consanguineous unions (Ringbauer et al., 2020) and inferring ancient population size (Fernandes et al., 2020). In addition, some preliminary insights into the sharing of long haplotypes between individuals associated to different archaeological cultures is providing evidence for recent biological connections between cultural groups. The added resolution offered by haplotype methods holds promise in not only elucidating patterns of close sharing and thereby identifying source populations, but also in identifying cultural transitions across which individuals may appear genetically similar in classical SNP-based methods (e.g. sharing of allele frequencies) but without long haplotype sharing, a pattern indicative of the arrival of a new population. However, maximal resolution will be obtained only when haplotype-based methods are combined with denser sampled archaeogenetic datasets, in which case previously identified turnovers will be refined and new turnovers detected.

Conclusion

The deluge of archaeogenetic data of the last decade has provided great insights into human (pre)history. With improving laboratory and computational methods, it is likely that sampling densities and resolution will grow, providing new perspectives on the history of our species. The insight gained into past distribution of human biological and pathogen diversity is also likely to continue to provide important insights into modern disease, therapeutics, and policies to manage and contains outbreaks and pandemics.

9. References for Introduction and Discussion sections

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10. Summary

The last thirty years have seen the field of ancient DNA mature into a robust scientific inquiry, revealing new and important insights into the last million years of natural history on Earth. It has transformed our understanding of the human past by uncovering new human species, admixture with archaic hominins, unexpected large-scale migrations, the presence and evolution of human diseases, as well as informing about the domestication processes of species important to humans. This thesis applies ancient DNA methods to better understanding the population history and social structure of Neolithic to Bronze Age Europe.

Manuscript A presents a large and densely sampled genetic time transect through Bohemia, spanning the Neolithic, Eneolithic and Early Bronze Age. By reporting 206 new individuals, and combining this with 65 previously published individuals, this study (along with the 77 new individuals reported in manuscript B) makes Bohemia one of the most densely sampled regions for ancient human DNA in the world. New insights into the population history of central Europe are gained, including the presence of previously unappreciated large demographic changes between genetically similar groups. Individuals of the Funnelbeaker and Globular Amphora cultures are shown to have likely been newcomers to Bohemia. The increased sampled sizes from the 3rd millennium BCE allow the cultural groups therein to be split into early and late phases, allowing the origin as well as development of each group to be studied. Early Corded Ware individuals are found to be genetically diverse and not descended from known Yamnaya groups, instead carrying added Eastern Hunter Gatherer-like ancestry. Four females without Yamnaya-like ancestry are found buried in early Corded Ware cultural contexts, suggesting the assimilation of pre-Corded Ware people into Corded Ware contexts was a sex-biased process. Thereafter, a genetic shift between early and late Corded Ware individuals is detected, implying these cultural groups were not genetically isolated through time. This includes a sharp reduction in Y-chromosome diversity, possibly reflecting a new social organisation. Bell Beaker individuals are shown to have carried more Neolithic-like ancestry compared to Corded Ware individuals, and their appearance in Bohemia is associated with a complete turnover and collapse to a single Y-chromosome lineage. No pre-Bell Beaker Y chromosomes are detected in Bell Beaker males, despite sometimes being buried at the same site. Individuals of the Early Bronze Age Únětice culture are shown to have had a large (≥40%) genetic contribution ultimately originating from the northeast, likely accompanied by a new social organisation.

Manuscript B presents a multi-disciplinary investigation of a densely (almost completely) sampled Early Bronze Age Únětice cemetery from Mikulovice, eastern Bohemia. The community's location on the Amber road, a north-south ancient trade route connecting the Baltic and Mediterranean seas along with the impressive amount of demonstrably exotic grave goods (including Baltic amber) provides a unique opportunity to study how the interconnectedness of the Early Bronze Age was manifested or sustained at the level of local communities. Through anthropological, stable isotope, and genetic data from 92 individuals, inferences about the community's kinship, social organisation, and patterns of mobility are made. Despite the presence of a surprisingly low incidence of stable isotope outliers (~10% non-locals), the community buried at Mikulovice was genetically homogeneous, suggesting that non-locals likely came from geographically not too distant regions. The analysis of pedigrees reveals a high incidence of missing (unsampled) individuals (~50%), suggesting

that Mikulovice was part of a wider community where these individuals may have been laid to rest. The data reveals a higher incidence of males staying in the community, consistent with a higher degree of patrilocality compared to matrilocality. Interestingly, three males who are biologically well connected to the Mikulovice cemetery (each have 5+ relatives at the site including parents and/or grandparents) show evidence of childhood mobility, likely spending a portion of their upbringing northeast of Bohemia, possibly close to the source of the Baltic amber found in Mikulovice. These results challenge the notion that stable isotope outliers are always complete foreigners to the communities in which they were buried, and instead suggest that people in the Early Bronze Age may have moved between regions, spending extended periods of their lives in different places.

Manuscript C presents the largest investigation of ancient hepatitis B virus (HBV) genetic diversity to date. By drastically increasing the sample size of ancient HBV, including the earliest HBV strains sampled to date (~10,000 years old), new insights into the origin, evolution, and spread of HBV are gained. Although it has been suggested that most human pathogens arose through close contact with domesticated animals after the transition to agriculture, Manuscript C provides evidence for the presence of HBV in hunter-gatherers. HBV strains common in European hunter-gatherers were subsequently replaced by a strain (WENBA strain) which arrived with Neolithic farmers, a genetic replacement which is also mirrored in the general replacement of hunter-gatherer-associated human autosomal ancestry by farmer-associated ancestry from south-eastern Europe and Anatolia. This WENBA strain spread as far east as present-day Moscow and Samara, regions which the traditional "Neolithic package" (as attested in central and western Europe) did not reach, suggesting spread via horizontal transfer through sporadic contacts between farmers and hunter-gatherers. Following its introduction with the arrival of agriculture in Europe (~5,500 BCE), the WENBA lineage was the dominant HBV lineage in Europe for 4,000 years until the 2nd millennium BCE which saw this lineage replaced by new lineages from the east, a genetic shift coinciding with the Late Bronze Age collapse and possibly a large population turnover.

Manuscript D presents a new capture assay for enriching Y-chromosomal DNA fragments in ancient DNA extracts. The Y-Mappable Capture Assay (YMCA) is shown to enrich for and provide more data on Y-chromosomal variation than shotgun sequencing or "1240K" capture. This added resolution is used to resolve the phylogeny of haplogroup H2, which reveals a phylogeographic pattern consistent with the two proposed routes of Neolithic expansion in Europe, Danubian and Mediterranean. The application of YMCA to many other ancient European males is likely to increase our understanding of the phylogeographic distribution of ancient Y-lineages, uncover extinct lineages, aid in pedigree reconstructions, and help retrieve more information from poorly preserved male samples.

Overall, this thesis reveals new insights into the population history and social structure of Neolithic to Bronze Age Europe. New insights have been primarily obtained through denser sampling, allowing for finer population structure to be revealed, thereby demonstrating the potential and importance of closing sampling gaps in the archaeogenetic record of ancient Europe.

11. Zusammenfassung

Innerhalb der letzten dreißig Jahre entwickelte sich die Erforschung der alten DNS (engl. *ancient DNA*) in eine selbständige und anerkannte Wissenschaft, welche seitdem neue und bedeutende Einblicke in die letzten eine Millionen Jahre Naturgeschichte unserer Erde ermöglicht. So revolutionierte sie nicht nur unser Verständnis der Menschheitsgeschichte durch die Entdeckung neuer Menschenarten, dem Nachweis der Vermischung verschiedener ausgestorbener Arten der Gattung *Homo*, und der Rekonstruktion massiver Wanderungsbewegungen in der Vorgeschichte, sondern gewährte ebenfalls auch grundlegende Einsichten in den Domestikationsprozess unserer heutigen Nutztiere als auch in das Vorkommen sowie die Evolution verschiedener Krankheitserreger.

In der vorliegenden Dissertationsschrift wird aufgezeigt, wie alte DNS eingesetzt werden kann, um unser Verständnis der Populationsgeschichte und Sozialstruktur Europas vom Neolithikum bis in die Bronzezeit maßgeblich zu verbessern und zu erweitern.

Manuskript A behandelt einen großangelegten, durch dichte Beprobung hochaufgelösten Zeittranssekt durch das Neolithikum, das Äneolithikum, und die ältere Bronzezeit in Böhmen. Insgesamt wurde alte DNS von 206 Individuen gewonnen und mit der 65 weiterer, bereits publizierter Individuen aus Böhmen analysiert. Summiert mit den 77 Individuen, welche in Manuskript B vorgestellt werden, macht dies Böhmen in Bezug auf alte DNS zu einer der am umfangreichsten untersuchten Regionen der Welt. Daraus ergeben sich neue Einblicke in die Populationsgeschichte Mitteleuropas, unter anderem in bis jetzt unerkannte jedoch substantielle demographische Umwälzungen zwischen genetisch nahe verwandten Populationen. So wird aufgezeigt, dass die Träger der Trichterbecherkultur (engl. *Funnelbeaker culture*) und Kugelamphorenkultur (engl. *Globular Amphora culture*) ursprünglich nicht auf dem Gebiet des heutigen Böhmens heimisch waren, sondern dorthin einwanderten. Dank der großen Stichprobe aus dem dritten Jahrtausend v. Chr. ist es nun erstmals auch möglich, Kulturgruppen in ältere und jüngere Phasen einzuteilen, um so sowohl den genetischen Ursprung als auch die demographische Entwicklung dieser Gruppierungen zu untersuchen. Es wird gezeigt, dass die Zusammensetzung der älteren Schnurkeramischen Kultur (engl. *Corded Ware culture*) genetisch überaus divers war, und dass ihre Träger nicht von einer der bekannten Jamnaja-Kulturgruppen (engl. *Yamnaya culture*) abstammten, wie ein erhöhter Anteil östlichen Jäger- und Sammler-Erbguts (engl. *Eastern Hunter-Gatherers*) belegt. Weiterhin zeigten vier weibliche Individuen, die im materiellen Kontext der frühen Schnurkeramik bestattet wurden, keinerlei Jamnaja-ähnliches Erbgut, was auf Geschlechterungleichtheit im Assimilationsprozess der vorschnurkeramischen Bevölkerung in die schnurkeramische Gesellschaft hindeutet. Am Übergang der älteren zur jüngeren Schnurkeramischen Kultur wird schließlich ein genetischer Umbruch nachgewiesen, welcher Austausch mit umliegenden Populationen suggeriert. Dieser Umbruch äußert sich vor allem in einer deutlichen Reduktion der Y-chromosomalen Diversität, was möglichweise auf einen Wechsel in der sozialen Ordnung zurückzuführen ist. Die zeitlich nachfolgenden Träger der Glockenbecherkultur (engl. *Bell Beaker culture*) weisen deutlich mehr von der neolithischen Bevölkerung Europas abstammendes Erbgut auf als die vorausgehenden Individuen der Schnurkeramischen Kultur. Entsprechend zeigt sich, dass die Ankunft und Ausbreitung der Glockenbecherkultur in Böhmen mit einem vollständigen Umsturz der Y-chromosomalen Vielfalt und deren Kollaps zu einer einzigen Y-chromosomalen Linie einhergeht. In keinem der untersuchten männlichen Glockenbecherkultur-Individuen konnten Y -Chromosom-Linien nachgewiesen

werden, die mit Kulturgruppen vor der Ankunft der Glockenbecher assoziiert werden, obgleich in einigen Fällen Kontinuität in der Nutzung der Gräberfelder besteht. Schließlich, mit dem Beginn der frühen Bronzezeit deutet sich vermutlich ein erneuter Wandel der Gesellschaft an, da Träger der frühbronzezeitlichen Aunjetitzer-Kultur (engl. *Unetice culture*) große Teil ihres Erbmaterials (≥40%) von einer Population beziehen, deren Herkunft im Nordosten Europas verortet wird.

In Manuskript B wird die multidisziplinäre Untersuchung eines nahezu vollständig beprobten frühbronzezeitlichen Gräberfeldes der Aunjetitzer-Kultur in Mikulovice, Ost-Böhmen, vorgestellt. Da sich diese Fundstelle an der Bernsteinstraße (engl. *Amber road*) befindet, einem von Norden nach Süden verlaufenden Handelsweg, welcher den mediterranen Raum mit dem Baltikum verband, birgt sie einzigartiges Potential für die Untersuchung wie sich lokale Gruppierungen während der frühen Bronzezeit räumlich vernetzten und Verbindungen instand hielten. Die Angebundenheit Mikulovices an dieses Handelsnetzwerk zeigt sich hierbei deutlich durch die beeindruckende Fülle exotischer Grabbeigaben, unter anderem baltischen Bernsteins. Unter Zuhilfenahme physisch-anthropologischer, isotopengeochemischer, und genetischer Daten von 92 Individuen wurden Rückschlüsse auf die Verwandtschaftsbeziehungen, Gesellschaftsordnung, und individuelle Mobilität innerhalb der Gemeinschaft gezogen. Wider Erwarten stellt sich die in Mikulovice bestatte Gemeinschaft als genetisch überaus homogen heraus, obgleich Analysen stabiler Isotopen bis zu 10% nicht-lokale Individuen nahelegen. Dies deutet daraufhin, dass jene Auswärtigen aus nicht allzu weit entfernten Regionen stammen mussten. Weiterhin ergab eine Untersuchung der Stammbäume eine große Anzahl fehlender, d.h. nicht beprobter Individuen (~50%). Dieser Umstand impliziert, dass Mikulovice Teil einer größeren Gemeinschaft war, die ihre Verstorbenen mutmaßlich an verschieden Orten bestattete. Ebenfalls wird ein mehr patrilokaler als matrilokaler Charakter der Gesellschaft durch die neuen Daten bestätigt, welcher sich in dem höheren Anteil in der Gemeinschaft verbleibender männlicher Individuen zeigt. Interessanterweise jedoch weisen gerade drei männliche Individuen Anzeichen von Mobilität in der Kindheit auf. Diese drei Individuen sind mit zahlreichen anderen auf dem Gräberfeld Bestatteten verwandt (jedes dieser Individuen hat zumindest 5 Verwandte in der Fundstelle, darunter Elter und/oder Großeltern), verbrachten aber ihre Kindheit in Nordost Böhmen, möglicherweise nahe der Quelle des in Mikulovice gefundenen baltischen Bernsteins. Diese Beobachtung steht in klarem Gegensatz zur gängigen Meinung, basierend auf Isotopen-Analysen identifizierte *Outlier*-Individuen müssten stets Fremde in der Gemeinschaft sein, in der sie begraben wurden. Vielmehr impliziert dies ein hohes Maß an Mobilität in der frühbronzezeitlichen Bevölkerung, und dass Individuen sich zwischen Regionen bewegten und längere Teile ihres Lebens an unterschiedlichen Orten verbrachten.

Manuskript C behandelt die bis dato umfangreichste Untersuchung prähistorischer Hepatitis-B-Viren (HBV) und ihrer genetischen Vielfalt, um den Ursprung, die Evolution, und die Ausbreitung dieses Erregers zu beleuchten. Hierfür wurde nicht nur die bestehende Stichprobe prähistorischer Hepatitis-B-Viren substanziell vergrößert, sondern auch der bis jetzt älteste Erregerstamm mit einem Alter von rund 10.000 Jahren rekonstruiert. Obgleich der Ursprung der meisten menschlichen Pathogene auf den nahen Kontakt zwischen Mensch und Nutztier nach der Adaptation von Ackerbau und Viehzucht zurückgeführt wird, wird in diesem Manuskript jedoch das Vorkommen von HBV bereits in Wildbeuter-Populationen nachgewiesen. Die in den Jäger- und Sammer-Populationen Europas nachgewiesenen Erregerstämme wurden folglich von einem neuen Stamm verdrängt

(WENBA), welcher zusammen mit den neolithischen Ackerbauern nach Europe gelangte. Somit spiegelt die Verdrängung des Wildbeuter-Erregerstammes durch den Neolithischen Erregerstamm den im menschlichen Genom stattfindenden Austausch des Jäger- und Sammler-Erbguts durch das Erbgut der aus Anatolien stammenden frühen Ackerbauern wider.

Die Verbreitung dieses WENBA-Stammes erstreckt sich im Osten bis zum heutigen Moskau und Samara, und somit in Regionen, in welche das traditionell angenommene „Neolithische Paket" im Gegensatz zu Mittel- und Westeuropa nicht vordringen konnte. Daraus ist zu schließen, dass sich der Erreger durch sporadischen Kontakt zwischen Ackerbauern und Wildbeutern horizontal ausbreitete. Nach seiner Einführung zu Beginn des Neolithikums zusammen mit Ackerbau und Viehzucht war der WENBA-Stamm für rund 4.000 Jahre die vorherrschende HBV-Linie in Europa, wurde jedoch im zweiten Jahrtausend v. Chr. selbst von einem neuen Erregerstamm aus dem Osten verdrängt. Auch dieser Prozess koinzidiert mit einem gesellschaftlichen Umbruch, dem sogenannten „Zusammenbruch der Bronzezeit" (engl. *Late Bronze Age collapse*), welcher gemeinhin ebenfalls mit tiefgreifenden demographischen Umwälzungen assoziiert wird.

In Manuskript D wird eine neue Methode für die Zielregionen-Anreicherung Y-chromosomaler DNS-Fragmente innerhalb von Extrakten alter DNS vorgestellt. Das Y-Mappable Capture Assay (YMCA) ermöglicht nachweislich die umfangreichere Rückgewinnung Y-chromosomaler Variation als die bekannte Schrotschuss-Sequenzierung (engl. *Shotgun sequencing*) oder „1240k"-Zielregionenanreicherung (engl. *Capture*). Die gewonnene Auflösung Y-chromosomaler Diversität wurde genutzt, um die Phylogenie der Haplogruppe H2 zu rekonstruieren. Die Phylogeographie dieser Haplogruppe rekapituliert die von archäologischer Seite hypothetisierten zwei Ausbreitungswege der neolithischen Expansion nach Europa, die Mittelmeer-Route (engl. *Mediterranean route*) und die Donau-Route (engl. *Danubian route*). Die Anwendung des YMCA auf weitere prähistorische männliche Individuen wird in Zukunft zum Verständnis der Phylogeographie weiterer Y-Chromosomaler Haplogruppen beitragen, so zum Beispiel beim Nachweis ausgestorbener Linien, oder auch die Rekonstruktion von Stammbäumen erleichtern. So ist es nun möglich selbst aus schlecht erhaltenem männlichen Fundmaterial zusätzliche genetische Information zu gewinnen.

Zusammenfassend gewährt diese Dissertation neue Einblicke in die Populationsgeschichte und Sozialstruktur Europas während des Neolithikums und der Bronzezeit. Diese Einblicke wurden vor allem durch Ausweitung der Beprobung gewonnen, wodurch subtile und bisher unerkannte Populationstrukturen aufgedeckt werden konnten. Dies beweist das Potential einer gleichmäßigen Beprobung und die Dringlichkeit, bestehende Lücken im archäogenetischen Befund des prähistorischen Europas zu schließen.

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13. Declaration of honour

NAME: Luka Papac
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Declaration of honour

Hereby I declare
(a) that I am aware of the applicable doctoral regulations,
(b) that I have written the doctoral thesis myself and that I have not taken any text sections from another author or from my own examination papers without indicating them and that I have indicated all tools and sources used by myself in this work,
(c) that I have mentioned all persons who have supported me in the selection and evaluation of the material as well as in the production of the manuscript,
(d) that I have not used the assistance of a commercial doctoral advisor and that third parties have neither directly nor indirectly received monetary benefits from me for work that is related to the content of the submitted doctoral thesis,
(e) that I have not yet submitted this doctoral thesis as an examination paper for an academic examination,
(f) that I have submitted neither the same thesis nor an essentially similar thesis, nor a different thesis as a doctoral thesis at another university.

Date: 23/06/2021

Name / Signature

Luka Papac

14. Eigenständigkeitserklärung

Entsprechend §5 Abs. 4 der Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena, erkläre ich, dass mir die geltende Promotionsordnung der Fakultät bekannt ist. Ich bezeuge, dass ich die vorliegende Dissertation selbst angefertigt habe und keine Textabschnitte eines Dritten oder eigener Prüfungsarbeiten ohne Kennzeichnung übernommen und alle von mir benutzten Hilfsmittel, persönliche Mitteilungen sowie Quellen in meiner vorliegenden Arbeit angegeben habe. Zudem habe ich alle Personen, die mir bei der Auswahl und Auswertung sowie bei der Erstellung der Manuskripte unterstützt haben, in der Auflistung der Manuskripte und den entsprechenden Danksagungen namentlich erwähnt. Zudem versichere ich, dass ich die Hilfe eines Promotionsberaters nicht in Anspruch genommen haben und auch Dritten von mir keine unmittelbaren sowie mittelbaren geldwerte Leistungen für Arbeiten, die im Zusammenhang mit dieser Dissertation stehen, erhalten haben. Die vorliegende Promotion wurde zuvor weder für eine staatliche oder andere wissenschaftliche Prüfung eingereicht, also auch einer anderen Hochschule als Dissertation vorgelegt.

Jena, den 23.06.2021

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Luka Papac

15. Compliance with legal requirements

Luka Papac, Max Planck Institute for the Science of Human History

I hereby confirm that I have complied with the legal requirements for animal protection, genetic engineering and species and biotope protection in my work “Tracking population history, social structure and intergroup exchange in Neolithic to Bronze Age Europe using ancient human and virus genomes.”

Jena, 23/06/2021 .....