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#### Supporting Online Material

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Materials and Methods

Fig. S1

References

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# Ancient DNA from the First European Farmers in 7500-Year-Old Neolithic Sites

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The ancestry of modern Europeans is a subject of debate among geneticists, archaeologists, and anthropologists. A crucial question is the extent to which Europeans are descended from the first European farmers in the Neolithic Age 7500 years ago or from Paleolithic hunter-gatherers who were present in Europe since 40,000 years ago. Here we present an analysis of ancient DNA from early European farmers. We successfully extracted and sequenced intact stretches of maternally inherited mitochondrial DNA (mtDNA) from 24 out of 57 Neolithic skeletons from various locations in Germany, Austria, and Hungary. We found that 25% of the Neolithic farmers had one characteristic mtDNA type and that this type formerly was widespread among Neolithic farmers in Central Europe. Europeans today have a 150-times lower frequency (0.2%) of this mtDNA type, revealing that these first Neolithic farmers did not have a strong genetic influence on modern European female lineages. Our finding lends weight to a proposed Paleolithic ancestry for modern Europeans.

Agriculture originated in the Fertile Crescent of the Near East about 12,000 years ago, from where it spread via Anatolia all over Europe (1). It has been widely suggested that the global expansion of farming included not only the dispersal of cultures but also of genes and languages (2). Archaeological cultures such as the Linear pottery culture (*Linearbandkeramik* or LBK) and Alföldi Vonaldiszes Kerámia (AVK) mark the onset of farming in temperate regions of Europe 7500 years ago (3). These early farming cultures originated in Hungary and Slovakia, and the LBK then spread rapidly as far as the Paris Basin and the Ukraine (4, 5). The remarkable speed of the LBK expansion within a period of about 500 years, and the general uniformity of this archaeological unit across

a territory of nearly a million square kilometers (Fig. 1), might indicate that the spread was fueled to a considerable degree by a migration of people (6–8). On the other hand, a number of archaeological studies suggest that local European hunter-gatherers had shifted to farming without a large-scale uptake of genes from the first farmers (9–11). Genetic studies carried out on modern Europeans have led to conflicting results, with estimates of Neolithic input into the present population ranging from 20 to 100% (12–20). A theoretical simulation study by Currat and Excoffier (21) has recently suggested a minor contribution, clearly less than 50%, and possibly much less. Conclusive ancient DNA studies on skeletons of the first European farmers have so far not been published to our knowledge.

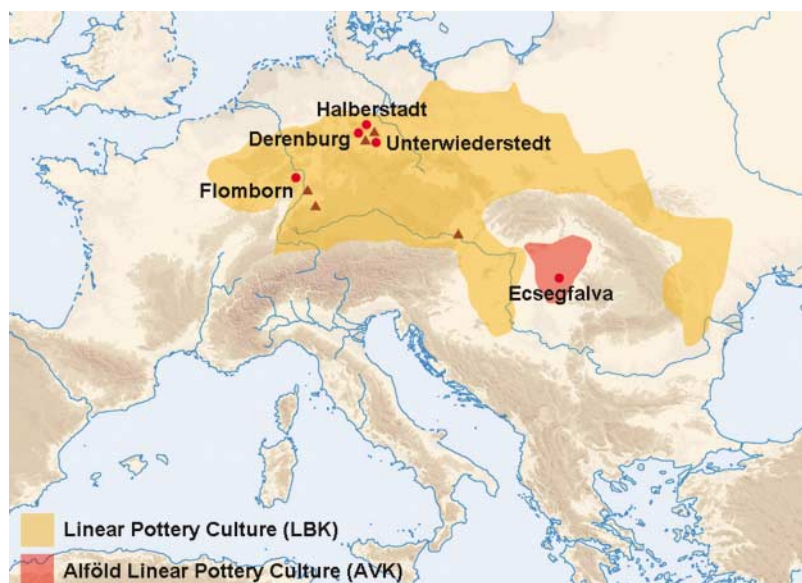
To resolve the question regarding the extent of the Neolithic female contribution to the present European population, we collected 57 Neolithic skeletons from 16 sites of the LBK/AVK culture from Germany, Austria, and Hungary. These include well-known archaeological sites such as Flomborn, Schwetzingen, Eilsleben, Asparn-Schletz, and several new excavations; for example, from Halberstadt and Derenburg Meerestieg II. All human remains were dated to the LBK or AVK period (7500 to 7000 years ago) on the basis of associated cultural finds. We extracted DNA from bone and teeth from the morphologically well-preserved individuals, and we amplified nucleotide positions (nps) 15997–16409 [see supporting online material (22)] of the mitochondrial genome with four overlapping primer pairs. In addition, we typed a number of coding-region mtDNA polymorphisms, which are diagnostic for major branches in the mtDNA tree (22).

From a total of 57 LBK/AVK individuals analyzed, 24 individuals (42%) revealed reproducibly successful amplifications of all four primer pairs from at least two independent extractions usually sampled from different parts of the skeleton. Eighteen of the sequences belonged to typical western Eurasian mtDNA branches; there were seven H or V sequences, five T sequences, four K sequences, one J sequence, and one U3 sequence (table S1). These 18 sequences are common and widespread in modern Europeans, Near Easterners, and Cen-

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**Fig. 1.** Geographic range of the first Central European farmers. The orange and red areas indicate the widest distribution of the earliest Neolithic farming cultures LBK and AVK after 7500 years before the present. Circles represent sites with N1a haplotypes, and triangles represent sites with other haplotypes. Names are given for N1a sites only. For details on the archaeological sites, see table S3.

**Table 1.** mtDNA sequences of the six Neolithic N1a types. Sequences are presented as variant nucleotide positions relative to the Cambridge Reference Sequence (31). Nucleotide positions are given, less 16000.

Individual	ID no.	mtDNA sequence 15997–16409
Derenburg 1	DEB1	147.A 172.C 223.T 248.T 355.T
Derenburg 3	DEB3	147.A 172.C 223.T 248.T 320.T 355.T
Halberstadt 2	HAL2	086.C 147.A 172.C 223.T 248.T 320.T 355.T
Flomborn 1	FLO1	147.A 172.C 223.T 248.T 320.T 355.T
Unterwiederstedt 5	UWS5	129.A 147.A 154.C 172.C 223.T 248.T 320.T 355.T
Ecsegfalva 1	ECS1	147.A 172.C 189.C 223.T 248.T 274.A 355.T

tral Asians, and thus these 18 lineages lack the detailed temporal or geographic discrimination required to test the hypotheses we are examining, even though some of them have previously been suggested to be of Neolithic origin on the basis of modern DNA studies (15). We therefore concentrated on the mtDNA types identified in the other six individuals.

The most striking result is that 6 of the 24 Neolithic skeletons are of the distinctive and rare N1a branch. For verification, we sequenced 517 clones derived from independent extractions from different parts of the six individuals. All six showed the suite of mutations characteristic of the N1a lineage. Five of these six individuals display different N1a types, whereas Flomborn 1 and Derenburg 3 show identical N1a types (Table 1).

The observed distinct N1a types rule out the possibility of contamination with modern samples, which can be a problem in ancient human DNA studies. It is implausible that the five types are from five different modern contaminants, because the frequency of this type today is very low anywhere in the world, at about 0.2% (23–25) (fig. S1). It is also unlikely that the sequence variations seen within the five N1a types are the result of random postmortem DNA damage

(26, 27), because three out of six sequence types that we have identified precisely match modern sequences previously published in the literature (table S2 and supporting references); finally, two further N1a types (HAL2 and UWS5) precisely fit into predicted but previously unobserved ancestral nodes in the N1a phylogeny (Fig. 2), underlining the authenticity of the ancient DNA.

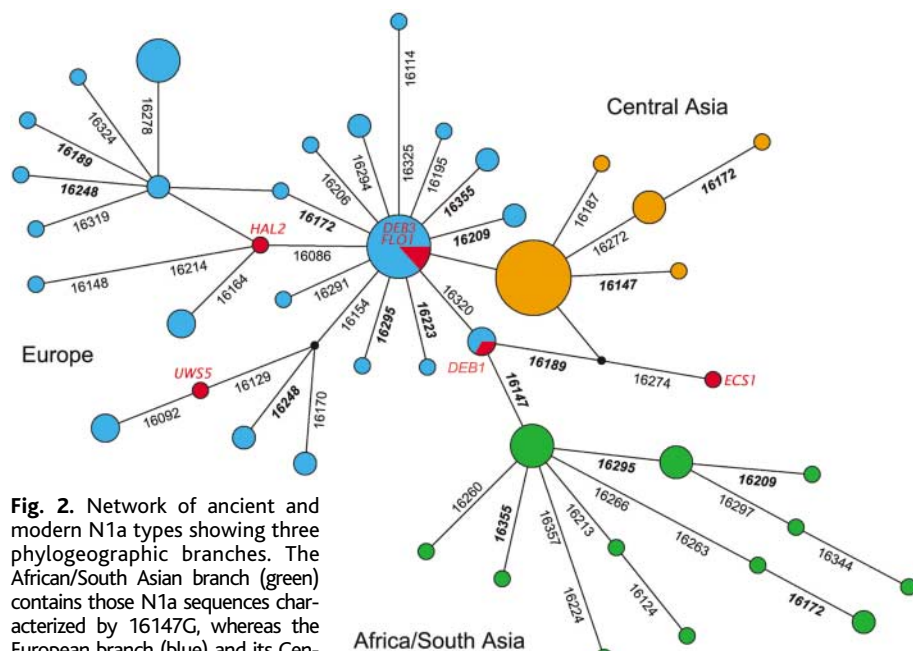
The high frequency of our Neolithic N1a lineages is not a local phenomenon but is widespread in the LBK area: Independently sampled locations in Hungary and Germany, over 800 km apart, each yielded one or more N1a types (Fig. 1). The modern geographic spread of N1a types partly reflects the Neolithic situation, albeit at a much lower modern frequency: All Neolithic LBK types fall into the “European” N1a sub-branch, and this sub-branch today is rare but widespread in Europe and adjacent parts of Asia and North Africa (Fig. 3). The AVK sample ECS1 shows 16189C, which is characteristic of the Central Asian branch, but in this case is plausibly a parallel mutation in the European branch, because position 16189 mutates much more rapidly than the conflicting 16320 position (28).

We next addressed the question of whether the 150-times lower frequency of N1a in modern

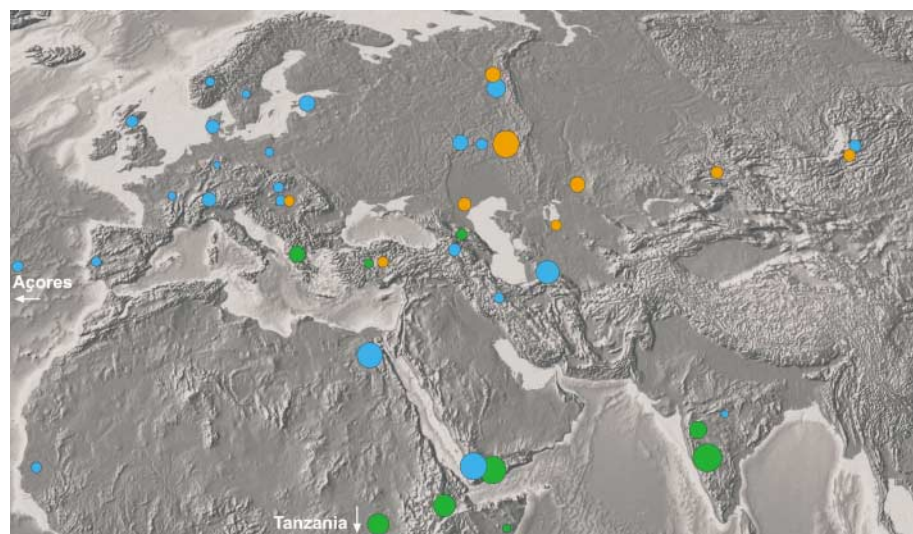
Europeans might be due to simple genetic drift over the past 7500 years. Given a frequency of N1a within our Neolithic sample of 25%, the frequency in the Neolithic LBK population is estimated to lie between 8% and 42% (95% confidence interval, based on binomial standard error). Even the lower limit of 8% contrasts markedly with an N1a frequency of 0.2% (5 in 2300) in modern mtDNA samples in the LBK area between the Paris Basin and Hungary. Qualitatively, modern Europeans therefore do not appear to be maternally descended from the first farmers. However, there remains a possibility that modern European maternal lineages are descended from the early farmers but that the N1a type has been lost during the past 7500 years through genetic drift. We therefore applied computer simulations to test whether the frequency of the Neolithic N1a types could have been drastically decreased by drift alone in the past 7500 years.

We simulated a scenario that would maximize the chance that N1a has been lost by genetic drift in the course of the past 7500 years. The simulation showed that we should observe at least 74 N1a’s out of the 2300 modern samples. In fact, 95% of the total runs ended showing between 119 and 259 N1a’s in the modern sample. Next, we allowed migration between the Neolithic population and the surrounding population per generation. The simulation showed that a migration rate of 1% per generation throughout 7425 years between the Neolithic population and the surrounding population is not enough to reduce the N1a percentage to the low value observed today, because only 5.5% of the total runs ended in <6 N1a’s in the modern sample.

These simulations reject the simple hypothesis in which modern Europeans are direct descendants of these first farmers and have lost N1a mainly by genetic drift. Hence the simulations confirm that the first farmers in Central Europe had limited success in leaving a genetic mark on the female lineages of modern Europeans. This is in contrast to the success of the Neolithic farming culture itself, which subsequently spread all over Europe, as the archaeological record demonstrates. One possible explanation is that the farming culture itself spread without the people originally carrying these ideas. This includes the possibility that small pioneer groups carried farming into new areas of Europe, and that once the technique had taken root, the surrounding hunter-gatherers adopted the new culture and then outnumbered the original farmers, diluting their N1a frequency to the low modern value. Archaeological research along the Western periphery of LBK and isotope studies of some of our sampled individuals seem to support the idea that male and female hunter-gatherers were integrated into the Neolithic communities (3, 10, 29). This hypothesis implies that N1a was rare or absent in Mesolithic Europeans, which may be a reasonable assumption given the rarity of the N1a type anywhere in the world (Fig. 3). An alternative hypothesis is a subsequent post-early-Neolithic population replacement in Europe,



**Fig. 2.** Network of ancient and modern N1a types showing three phylogeographic branches. The African/South Asian branch (green) contains those N1a sequences characterized by 16147A, whereas the European branch (blue) and its Central Asian subcluster (orange) are characterized by 16147A. The six early Neolithic DNA sequences are shown in red. Two of these ancient farmers (HAL2 and UWS5) fall into hitherto unsampled but predicted nodes, further confirming the authenticity of the ancient DNA. The Central Asian subcluster is at least 2500 years old, because the nodal Central Asian N1a type had been found in a Scytho-Siberian burial in the Altai region (30). Circles and pie-slice sizes are proportional to frequencies, and mutated nucleotide positions are shown along the branches.



**Fig. 3.** Modern geographic spread of the three N1a branches. Blue circles depict the European branch of N1a, orange circles the Central Asian branch of N1a, and green circles the African/South Asian branch. The three N1a branches are defined in the network of Fig. 2. The smallest circle size corresponds to a local frequency of 0.18%, and larger frequencies are indicated by proportionately larger circles.

eliminating most of the N1a types. Archaeological evidence for such an event is as yet scant.

The results from the Neolithic sample show that other mtDNA lineages considerably diluted the mtDNA pool of these early Neolithic populations, so that the frequency of N1a in modern Europeans is 150 times lower than in our sample of the first Central European farmers. This is incompatible with the idea that modern Central Europeans—and by implication other Europeans beyond the LBK/AVK area—derive their mater-

nal lineages purely from the earliest farmers of that region. Within the current debate on whether Europeans are genetically of Palaeolithic or Neolithic origin, and leaving aside the possibility of significant post-Neolithic migration, our data lend weight to the arguments for a Palaeolithic origin of Europeans.

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