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Genetic Discontinuity Between Local Hunter-Gatherers and Central Europe's First Farmers

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After the domestication of animals and crops in the Near East some 11,000 years ago, farming had reached much of central Europe by 7500 years before the present. The extent to which these early European farmers were immigrants or descendants of resident hunter-gatherers who had adopted farming has been widely debated. We compared new mitochondrial DNA (mtDNA) sequences from late European hunter-gatherer skeletons with those from early farmers and from modern Europeans. We find large genetic differences between all three groups that cannot be explained by population continuity alone. Most (82%) of the ancient hunter-gatherers share mtDNA types that are relatively rare in central Europeans today. Together, these analyses provide persuasive evidence that the first farmers were not the descendants of local hunter-gatherers but immigrated into central Europe at the onset of the Neolithic.

Europe has witnessed several changes in archaeological cultures since anatomically modern humans displaced the Neandertal population 30,000 to 40,000 years ago (*1*, *2*). Palaeolithic hunter-gatherers survived the Last Glacial Maximum (LGM) about 25,000 years ago in southern and eastern refugia (*3*) and resettled central Europe after the retreat of the ice sheets. With the end of the Ice Age at ~9600 B.C.E., their Mesolithic descendants or successors had recolonized large parts of the deglaciated northern latitudes (*4*, *5*). From around 6400 B.C.E., the hunter-gatherer way of life gave way to farming cultures in a transition known as the Neolithic Revolution (*6*). The extent to which this important cultural transition was mediated by the arrival of new peoples, and the degree of Mesolithic and early Neolithic ancestry in Europeans today, have been debated for more than a century (*7*–*10*). To address these questions directly, we obtained mitochondrial DNA (mtDNA) types from 22 central and northern European post-LGM hunter-gatherer skeletal remains (Fig. 1) and compared 20 of these (those for which full sequence information was available) to homologous mtDNA sequences from 25 early farmers (*11*, *12*) and 484 modern Europeans from the same geographic region (*13*). Our ancient sample spans a period from circa (ca.) 13,400 to 2300 B.C.E. and includes bones from Hohler Fels in the Ach valley (Late Upper Paleolithic) and Hohlenstein-Stadel in the Lone valley (Mesolithic). Extensive precautions were taken to ensure sequence authenticity (*14*), including extracting independent samples from different skeletal locations of the same individuals and examining remains only from high latitudes or cave sites with good biomolecular preservation.

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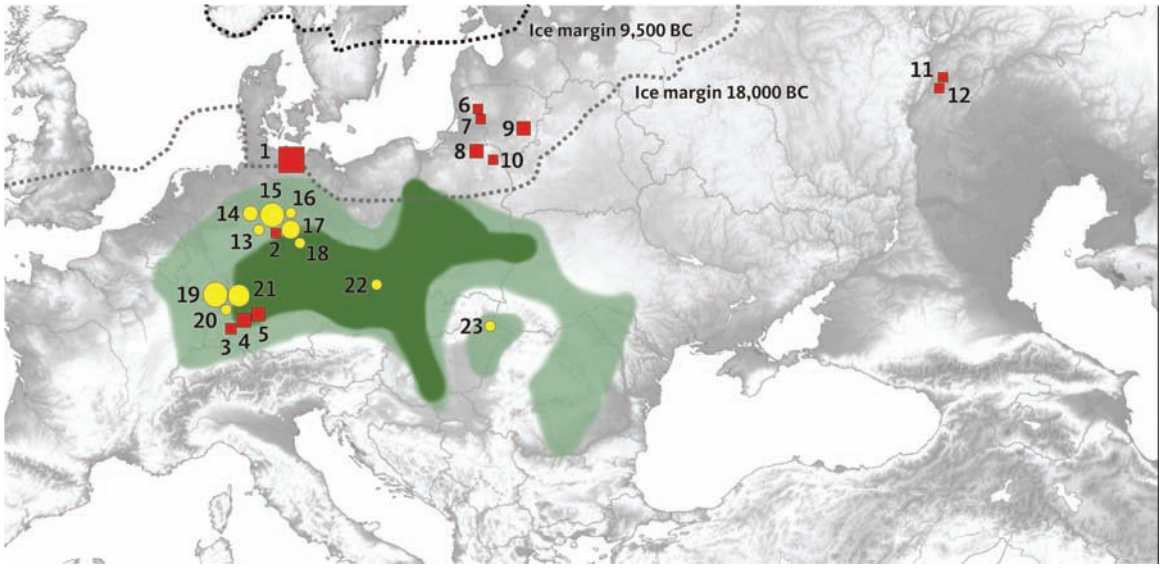

Fig. 1. mtDNA types from prehistoric samples of hunter-gatherers and farmers. The green shading represents the first farming areas [dark green: early LBK, 5650 to 5400 calibrated years B.C.E. (calBC); light green: LBK, 5400 to 4900 calBC] in central Europe, based on archaeological finds, whereas squares represent successfully analyzed Late Palaeolithic, Mesolithic, and Ceramist hunter-gatherers dating from 13,400 to 2300 B.C.E. The term "Neolithic" is sometimes applied to the Eastern European Ceramist culture because of their use of pottery, but this does not imply a farming economy (*21*). Previously analyzed (*11*, *12*) LBK farming sites are marked with circles for comparison. The area of each square or circle is proportional to the number of individuals successfully investigated. In red are labeled archaeological sites with one or more U4/U5 individuals; in yellow, sites with other mtDNA types, highlighting the specificity of U types in the prehistoric hunter-gatherers. The sites are as follows: 1, Ostorf; 2, Bad Dürrenberg; 3, Falkensteiner Höhle; 4, Hohler Fels; 5, Hohlenstein-Stadel; 6, Donkalnis; 7, Spiginas; 8, Dudka; 9, Kretuonas; 10, Drestwo; 11, Chekalino; 12, Lebyazhinka; 13, Unseburg; 14, Unterwiederstedt; 15, Derenburg/Meerenstieg; 16, Eilsleben; 17, Halberstadt; 18, Seehausen; 19, Flomborn; 20, Vaihingen an der Enz; 21, Schwetzingen; 22, Asparn/Schletz; 23, Ecsegfalva.

An analysis of the molecular variance (*15*) showed that our early farmers and hunter-gatherers were from two well-differentiated populations; the among-populations proportion of genetic variation (F_{ST}) = 0.163, $P < 10^{-6}$. To put this value into perspective, we compared a range of modern human populations, randomly sampling 20 individuals from each. The maximum F_{ST} value in all comparisons among eight modern European samples was 0.0327, and among 13 modern European, Middle Eastern, Indian, Chinese, Papua New Guinean, and Australian samples it was 0.133 (*14*). We also found that our modern European sample was significantly different from the early farmer (F_{ST} = 0.0580, $P = 10^{-5}$) and hunter-gatherer (F_{ST} = 0.0858, $P < 10^{-6}$) samples. To test whether these genetic differences can be explained under the null hypothesis of population continuity alone, we performed coalescent simulations across a wide range of ancestral population size combinations. We conservatively assumed a modern female effective population size of N_0 = 12,000,000 (one-10th of the current female population size of central and northern Europe) and two periods of exponential growth: the first after the Upper Paleolithic colonization of Europe 45,000 years ago of female effective population size N_{UP}, sampled from an ancestral African population of constant female effective size N_A = 5000; and the second after the Neolithic transition in central Europe 7500 years ago of effective population size N_N. We sampled sequences from each simulation according to the numbers (hunter-gatherer n = 20, early farmer n = 25, modern n = 484) and dates (Table 1) of the sequences presented here and found the proportion of simulated F_{ST} values that were greater than those observed ($P_{S>O}$) (*14*). By exploring all combinations of 100 values for N_{UP} (ranging from 10 to 5000) and 100 values for N_N (ranging from 1000 to 100,000), we found that the maximum $P_{S>O}$ value between hunter-gatherers and early farmers was 0.022 (for N_{UP} = 4960 and N_N = 1000), and the maximum $P_{S>O}$ value between hunter-gatherers and modern central Europeans was 0.028 (for N_{UP} = 3560 and N_N = 1000). Most $P_{S>O}$ values were considerably lower (Fig. 2). These results allow us to reject direct continuity between hunter-gatherers and early farmers, and between hunter-gatherers and modern Europeans.

When we considered continuity between early farmers and modern Europeans, we did identify ancestral population size combinations where $P_{S>O}$ > 0.05 (black shaded area in Fig. 2C). Thus, there are demographic conditions under which the observed genetic differences between early European farmers and modern Europeans can be explained by assuming population continuity. Those conditions include assuming N_N < 3000, an effective female population size that may be considered implausibly low and is certainly lower than the current archaeological census estimates of 124,000 (*16*). However, we note that (i) ancestral population sizes are notoriously difficult to estimate from archaeological data, and (ii) the relationship between effective and census population size is dependent on unknown factors, including mating systems and population substructure.

Most modern European mtDNA lineages can be assigned to one of the following clades or haplogroups: H, V, U (including K), J, or T, all deriving from clade R; or I, W, or X, the descendants of clade N. Although some subclades, such as U5, are fairly specific to Europe, most are shared with adjacent areas of Asia and North Africa and are of uncertain antiquity in Europe. We are therefore cautious about treating specific clades as markers of particular past population groups or demographic episodes (*17*). Nonetheless, it is intriguing to note that 82% of our 22 hunter-gatherer individuals carried clade U (14 U5, 2 U4, and 2 unspecified U types; Table 1). A high incidence of U types (particularly those belonging to the U5 subclade) in Stone Age Europeans has been inferred from modern mtDNA (*7*), but the frequencies found here are surprisingly high. Europeans today have moderate frequencies of U5 types, ranging from about 1 to 5% along the Mediterranean coastline to 5 to 7% in most core European areas, and rising to 10 to 20% in northeastern European Uralic speakers, with a maximum of over 40% in the Scandinavian Saami. U4 types show frequencies between 1 and 5% in most parts of Europe, with Western Europe at the lower end of this range and northeastern Europe and central Asia showing percentages in excess of 7% (*13*).

The diversity among the hunter-gatherer U types presented here, together with their continued presence over 11 millennia, and the fact that U5 is rare outside Europe, raises the possibility that U types were common by the time of the post-LGM repopulation of central Europe, which started around 23,000 years ago (3). In a previous study, we showed that the early farmers of central Europe carried mainly N1a, but also H, HV, J, K,

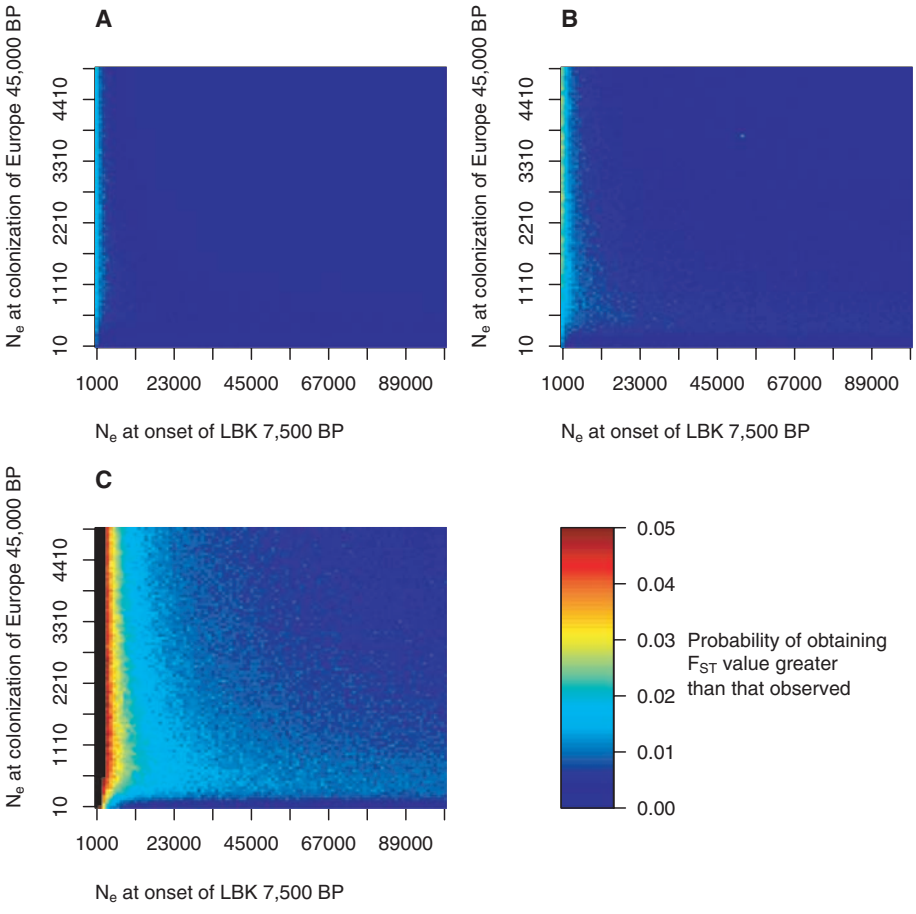

Fig. 2. Probabilities of obtaining observed genetic differences, as measured by F_{ST}, between (**A**) hunter-gatherers and LBK early farmers, (**B**) hunter-gatherers and modern Europeans, and (**C**) LBK early farmers and modern Europeans, across a range of assumed ancestral population size combinations. Two phases of exponential growth were considered, the first after the initial colonization of Europe 45,000 years ago, of assumed effective female population size N_{UP} (y axis), and ending when farming began in central Europe 7500 years ago, when the assumed effective female population size was N_N (x axis); and the second leading up to the present, when the assumed effective female population size is 12 million. The initial colonizers of Europe were sampled from a constant ancestral African population of 5000 effective females. The F_{ST} values are those observed from the data presented in this study. Black shaded areas indicate probabilities >0.05.

T, V, and U3 types (*11*, *12*). We found no U5 or U4 types in that early farmer sample. Conversely, no N1a or H types were observed in our hunter-gatherer sample, confirming the genetic distinctiveness of these two ancient population samples. This is particularly surprising as there is clear evidence for some continuity in the material culture between the central European Mesolithic and the earliest settlements of the Neolithic Linearbandkeramik culture (LBK) (*18*). Thus, it seems that despite the exchange of stone artifacts, genetic exchange between both groups, at least on the female side, was initially limited. The only exception is the site Ostorf (northern Germany), where two individuals carried haplogroup T2, which is also found in our LBK sample. We are cautious about interpreting this as a signature of local admixture (*17*), particularly because the hunter-gatherer and early farmer T2 types belong to different sublineages, but it is notable that Ostorf is culturally a Mesolithic enclave surrounded by Neolithic funnel-beaker farmers and is the only hunter-gatherer site where any non-U mtDNA types were observed (Table 1). Further sampling from such local contexts should shed light on the details of Mesolithic-Neolithic interactions after the arrival of farming. We note that any genetic exchange between hunter-gatherers and early farmers at this site would reduce the overall genetic differentiation between the two groups, so inclusion of this site has, if anything, a conservative effect on our conclusions regarding continuity.

Taken together, our results indicate that the transition to farming in central Europe was accompanied by a substantial influx of people from outside the region who, at least initially, did not mix significantly with the resident female hunter-gatherers. We accept that alternative, more complex demographic scenarios, such as strong local population structure and high group extinction and fission rates, might also explain our data. However, the ubiquity of U types in our hunter-gatherer samples is inconsistent with extensive population structuring and indicates that the demographic processes that shaped the observed patterns of genetic variation extend beyond the local scale.

The extent to which modern Europeans are descended from incoming farmers, their hunter-gatherer forerunners, or later incoming groups remains unresolved. The predominant mtDNA types found in the ancient samples considered in this study are found in modern Europeans, but at considerably lower frequencies, suggesting that the diversity observed today cannot be explained by admixture between hunter-gatherers and early

Table 1. Stone Age individuals and their mtDNA results. A, DNA of the archaeologists available for comparison; D, diagenetical analysis; M, multiple extractions and number of these; C, clones of the hypervariable segment 1 and number of these; N, positive amplification of nuclear DNA; Rf, restriction fragment length polymorphism analysis; SNP, single-nucleotide polymorphisms from the coding region of mtDNA obtained by means of multiplex amplification; BP, before the present; ca., circa. The mtDNA was sequenced from nucleotide position (np) 15997 to np 16409. mtDNA positions are numbered according to the revised Cambridge reference sequence (*22*), minus 16,000. Fourteen individuals did not yield results (table S1), whereas for two individuals the mtDNA sequences were not determined (n.d.) and thus not considered in the AMOVA analysis and simulations.

Country	Site, skeleton	Basis of dating*	Dating calBC*	Analyses	mtDNA sequence	Clade
Lithuania	Spiginas 4	GIN-5571: 7470 ± 60 BP	ca. 6350 calBC	A, M3, C109, Q, Rf	356c	U4
	Donkalnis 1	Cultural context	Mesolithic	A, D, M4, C79, N, Rf, SNP	192t 270t	U5b2
	Kretuonas 3	OxA-5926: 5580 ± 65 BP	ca. 4450 calBC	A, M4, C72, N, Rf, SNP	192t 270t	U5b2
	Kretuonas 1	OxA-5935: 5350 ± 130 BP	ca. 4200 calBC	A, M5, C56, N, Rf, SNP	192t 270t	U5b2
Poland	Dudka 2	^{14}C date on charcoal	ca. 3650 calBC	A, M3, C80, N, Rf	189c 270t	U5b1
	Dudka 3	Cultural context	4000-3000 calBC	A, M3, C127, Q, Rf	189c 265 g 270t	U5b1
	Drestwo 2	Ua-13085: 3805 ± 70 BP	ca. 2250 calBC	D, M4, C102, N, Rf	192t 256t 270t	U5a
Russia	Chekalino IVa	^{14}C date on shell Chekalino IVb	ca. 7800 calBC	A, D, M2, C83, Rf	192t 256t 270t 294t	U5a
	Lebyazhinka IV	^{14}C date on shell and cultural context	8000–7000 calBC	A, D, M2, C60, Rf	192t 241a/c 256t 270t 399 g	U5a1
Germany	Bad Dürrenberg 2	OxA-3136: 7930 ±90 BP	ca. 6850 calBC	A, D, M2, C 119, Rf	356c	U4
	Hohlenstein-Stadel, 5830a	ETH-5732: 7835 ± 80 BP	ca. 6700 calBC	M1, SNP	114a 192t 256t 294t 311c	U5a1
	Hohlenstein-Stadel, 5830b	ETH-5732: 7835 ± 80 BP	ca. 6700 calBC	M1, SNP	192t 270t	U5b2
	Hohler Fels, 49 Ib1 66	^{14}C dates on bone (H 5312-4907: 12,770 ± 110 BP; H 5119-4601: 13,085 ± 95 BP) and cultural context	Magdalenian ca. 13,400 calBC	M2, SNP	CRS	U
Germany	Hohler Fels, 10 Ic 405	^{14}C dates on bone (H 5312-4907: 12,770 ± 110 BP; H 5119-4601: 13,085 ± 95 BP) and cultural context	Magdalenian ca. 13,400 calBC	M2, SNP	n.d.	U
	Falkensteiner Höhle, FH	ETH-7615: 8185 ± 80 BP	ca. 7200 calBC	M2, SNP	n.d.	U5b2
	Ostorf SK28a	^{14}C dates and context	ca. 3200 calBC	A, M2, C18	224c 311c	K
	Ostorf SK8d	^{14}C dates and context	ca. 3200 calBC	A, M2, C16	270t	U5
	Ostorf SK35	^{14}C dates and context	ca. 3100 calBC	A, M2	270t	U5
	Ostorf SK12a	^{14}C dates and context	ca. 3000 calBC	A, M2	093y 126c 153a 294t	T2e
	Ostorf SK45a	^{14}C dates and context	ca. 3000 calBC	A, M2, C16	069t 126c	J
	Ostorf SK18	^{14}C dates and context	ca. 3000 calBC	A, M4	093c 126c 153a 294t	T2e
	Ostorf SK19	^{14}C dates and context	ca. 2950 calBC	A, M3	168t 192t 256t 270t 302 g	U5a

*Radiocarbon dates with laboratory numbers refer to direct dates of the skeleton and were calibrated with the program CalPal (*23*) on the basis of Intcal04. Corrections of reservoir effects were applied where identified.

farmers alone. If this is the case, then subsequent dilution through migration and admixture, after the arrival of the first farmers, would need to be invoked, implying multiple episodes of population turnover, which are not necessarily observable in the archaeological record. This, in turn, would mean that the classic model of European ancestry components (contrasting hunter-gatherers with early Neolithic farming pioneers) requires revision.

The geographic origin of the demographic processes that brought the early farmer mtDNA types to central Europe now becomes a major question. On the one hand, all of the early farmer remains analyzed here are associated with the LBK culture of central Europe. Based on ceramic typology, the LBK culture is thought to have originated in present-day western Hungary and southwestern Slovakia, with a possible predecessor in the southeast European Starçevo-Kris culture (*19*, *20*). These cultural source locations may provide the most plausible origins or routes for the geographic spread of the early farmers, considering that the LBK was the first major farming culture in central and northern Europe and is archaeologically attested to have disseminated over five centuries and covered nearly a million square kilometers. Alternatively, the farmers' mtDNA types may have an origin closer to the Neolithic core zone in southwestern Asia. Further ancient DNA analysis of early farmer samples from southeastern Europe and Anatolia will be required to resolve this question.

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Supporting Online Material

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Materials and Methods
Fig. S1
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Ribosomal Protein S6 Kinase 1 Signaling Regulates Mammalian Life Span

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Caloric restriction (CR) protects against aging and disease, but the mechanisms by which this affects mammalian life span are unclear. We show in mice that deletion of ribosomal S6 protein kinase 1 (S6K1), a component of the nutrient-responsive mTOR (mammalian target of rapamycin) signaling pathway, led to increased life span and resistance to age-related pathologies, such as bone, immune, and motor dysfunction and loss of insulin sensitivity. Deletion of *S6K1* induced gene expression patterns similar to those seen in CR or with pharmacological activation of adenosine monophosphate (AMP)–activated protein kinase (AMPK), a conserved regulator of the metabolic response to CR. Our results demonstrate that S6K1 influences healthy mammalian life span and suggest that therapeutic manipulation of S6K1 and AMPK might mimic CR and could provide broad protection against diseases of aging.

Genetic studies in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* implicate several mechanisms in the regulation of life span. These include the insulin and insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway and the mammalian target of rapamycin (mTOR) pathway, which both activate the downstream effector ribosomal protein S6 kinase 1 (S6K1) (*1*, *2*). Although the role of these pathways in mammalian aging is less clear, there is mounting evidence that IIS regulates life span in mice (*1*). Global deletion of one allele of the IGF-1 receptor (*Igf1r*), adipose-specific deletion of the insulin receptor (*Insr*), global deletion of insulin receptor substrate protein 1 (*Irs1*), or neuron-specific deletion of *Irs2*, all increase mouse life span (*1*). Life-span–extending mutations in the somatotropic axis also appear to work through attenuated IIS (*3*). *Igf1r* has also been implicated as a modulator of human longevity (*4*). However, the action of downstream effectors of IIS or mTOR signaling in mammalian longevity is not fully understood.

S6K1 transduces anabolic signals that indicate nutritional status to regulate cell size and

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