

Technologies, Wyss Institute, and an NSF Graduate Research Fellowship to D.B.G. Data can be accessed on the National Center for Biotechnology Information, NIH, Sequence Read Archive (SRA) (SRP029609). pGERC reporter can be obtained from AddGene (#47441). Accession numbers: The Project accession at the SRA is SRP029609. The sample accession is SRS477429. There are three experiments, one for DNA, one for

RNA, one for FlowSeq: RNA, SRX346948; DNA, SRX346944; and FlowSeq, SRX346268.

#### Supplementary Materials

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## 2000 Years of Parallel Societies in Stone Age Central Europe

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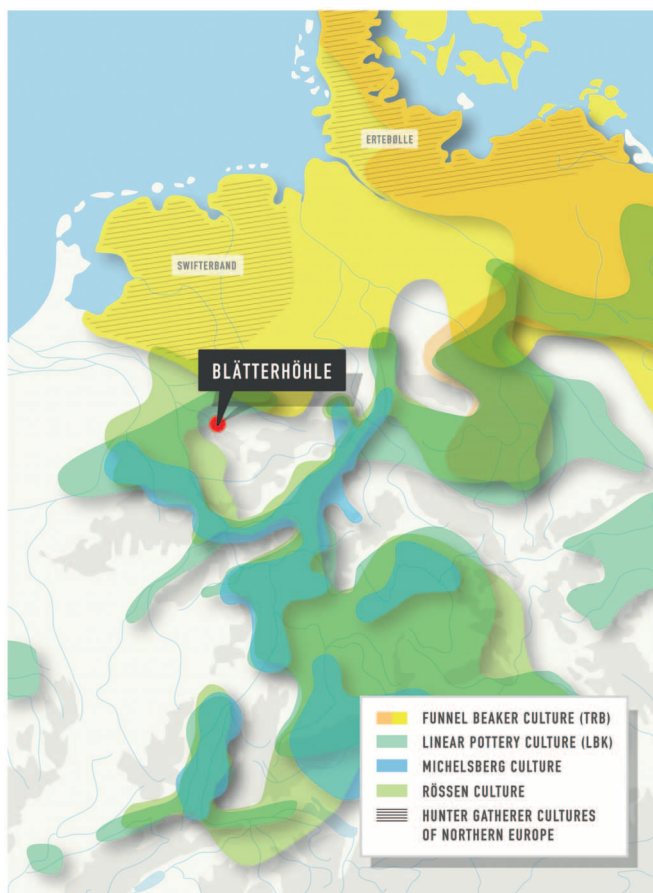
Debate on the ancestry of Europeans centers on the interplay between Mesolithic foragers and Neolithic farmers. Foragers are generally believed to have disappeared shortly after the arrival of agriculture. To investigate the relation between foragers and farmers, we examined Mesolithic and Neolithic samples from the Blätterhöhle site. Mesolithic mitochondrial DNA sequences were typical of European foragers, whereas the Neolithic sample included additional lineages that are associated with early farmers. However, isotope analyses separate the Neolithic sample into two groups: one with an agriculturalist diet and one with a forager and freshwater fish diet, the latter carrying mitochondrial DNA sequences typical of Mesolithic hunter-gatherers. This indicates that the descendants of Mesolithic people maintained a foraging lifestyle in Central Europe for more than 2000 years after the arrival of farming societies.

The Mesolithic-Neolithic transition marks a shift from a foraging to an agricultural way of life. It first appeared around 8500 BC in present-day southeastern Anatolia and Syria. About 3000 years later, this subsistence strategy reached Central Europe through the expansion of the Neolithic Linear Pottery culture (LBK). Whether the first European farmers descended from hunter-gatherers or migrated in from the Near East has been debated extensively in the archaeological literature. Over the last decade, a number of palaeogenetic studies have contributed substantially to current understanding of the Mesolithic-Neolithic transition in Europe [(1) and references therein]. Taken together, these findings strongly support a demic diffusion of early farmers into Central Europe, most likely originating in the southeast of the continent (2). Little is known about how long hunter-gatherers persisted in Central Europe, as there are no unambiguous signs of their presence in the archaeological record after the Early Neolithic. In this study, we present both ancient DNA and isotopic data, which, when combined, provide persuasive evidence for the prolonged coexistence of genetically distinct hunter-gatherer and farming groups over the course of the Neolithic in Central Europe.

Ancient DNA and sulfur, nitrogen, and carbon isotope ratios were analyzed from bones and teeth of 29 individuals from a burial cave site that contained around 450 remains from both Meso-

lithic hunter-gatherers and Neolithic individuals. The Blätterhöhle site is situated in Hagen, Germany (3) (Fig. 1), and because of its long and narrow geological structure, it is very likely that the human remains were deposited deliberately. Because the layers inside the cave have been disturbed by bioturbation, all samples used in this study were <sup>14</sup>C dated by accelerator mass spectrometry. The <sup>14</sup>C dates reveal two occupation phases ranging from 9210 to 8340 calibrated BCE (cal BC) (Mesolithic) and from 3986 to 2918 cal BC (Neolithic), respectively (4) (Table 1, table S1, and fig. S1).

We applied both a polymerase chain reaction, with subsequent Sanger sequencing, and a capture next-generation sequencing approach to establish partial or complete mitochondrial genomes. Out of 29 samples, 25 yielded reproducible mitochondrial hypervariable region I (HVRI) sequences (4) (Table 1 and tables S4 and S6). Complete mitochondrial genomes with coverage from 3.6× up to 39.8× were obtained for one Mesolithic and five Neolithic samples (4) (tables S4 and S6).



**Fig. 1. Geographic location of the Blätterhöhle cave site with schematic representation of the distribution of relevant archaeological cultures in Central and Northern Europe (27).**

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All five Mesolithic samples belong to the mitochondrial haplogroup (hg) U, in common with all previously typed pre-Neolithic hunter-gatherers of Central, Eastern, and Northern Europe (2, 5). Twelve of the Neolithic sequences also belong to hg U, whereas eight belong to other mitochondrial clades, such as hg H and J, as typically observed in early Neolithic farmers (1). Although U lineages are rare among early farmers, they appear at an unexpectedly high frequency [60%: 95% confidence interval (CI) = 38 to 78%] in our Late Neolithic Blätterhöhle sample. So at first glance, the Neolithic population of Blätterhöhle would appear to be a group of farmers with a high proportion of Mesolithic ancestry. However, dietary stable isotope evidence from the same specimens reveals a very different picture.

Three main clusters can be identified from the carbon and nitrogen isotopes plot (Fig. 2 and table S7): A first group, comprising all Mesolithic individuals, has particularly low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. This is consistent with a diet primarily of the wild fauna identified in the cave and similar to other inland Mesolithic sites (6, 7). The second group is from the Late Neolithic, and its isotope values reflect a terrestrial diet of herbivores, most likely domesticated animals. Their  $\delta^{15}\text{N}$  values are slightly enriched in  $^{15}\text{N}$  compared with the Mesolithic group, which may be explained by the cultural practice of manuring the pasture land where domesticated animals were raised (8). This kind of enriched  $\delta^{15}\text{N}$  is also observed in other Neolithic farming sites in Germany (9, 10). The third group also dates to the Late Neolithic, but, in contrast to groups 1 and 2, the isotope results point to a highly unusual diet, at least for the Neolithic period. In this group both carbon and nitrogen isotope values are very enriched, which suggests a diet low in plant and herbivore protein and high in freshwater fish (11, 12). The  $\delta^{34}\text{S}$  values strengthen this interpretation by showing their dietary protein came from freshwater sources (12) (Fig. 2B), whereas the Mesolithic hunter-gatherers and Neolithic farmers consumed terrestrial plants and animals.

Like the Mesolithic individuals of group 1, the mitochondrial DNA (mtDNA) sequences of the Neolithic freshwater fish consumers consist solely of U lineages, with none of the mitochondrial lineages typically observed in farmers (2). Thus, group 3 not only had a fisher-hunter-gatherer diet and lifestyle but also a signature of European Mesolithic hunter-gatherer ancestry. In contrast, the contemporaneous group 2 carried both mitochondrial lineages typically found in European Neolithic farmers and those found in European Mesolithic hunter-gatherers. As their isotope signature shows the typical pattern of individuals that consume domestic herbivores, they can be considered agriculturalists.

In summary, the results of  $^{14}\text{C}$  and stable isotope analysis, together with the DNA evidence, suggest that the Blätterhöhle individuals are sampled from three distinct populations: (i) Mesolithic hunter-gatherers, (ii) Neolithic farmers, and (iii) Neolithic fisher-hunter-gatherers (specializing in freshwater fish). The latter two notably date to the fourth mil-

lennium BC, which is around 2000 years after the introduction of farming to Central Europe.

Genetically, Blätterhöhle Neolithic fisher-hunter-gatherers appear rather similar to other Neolithic hunter-gatherers (table S9C) but exhibit a relatively high (albeit not significant) fixation index (or proportion of genetic variation due to population differentiation,  $F_{\text{ST}}$ ) value of 0.107 compared with the Mesolithic hunter-gatherers from the same cave site 5000 years before. At first sight, this would seem to cast doubt on a direct genetic continuity between them. However, coalescent simulations do not reject a model of population continuity under a very wide range of demographic parameters (4) (fig. S6A).

In another demographic model, we tested for continuity between Late Neolithic farmers and modern Europeans ( $F_{\text{ST}} = 0.016$ ) (table S9B). Although Bramanti *et al.* (2) found that population continuity between early (LBK) farmers and modern Europeans could be rejected, our coalescent simulations provide no evidence to reject continuity between the later Neolithic farmers (table S8) and modern Europeans (4) (table S8 and fig. S6B). This indicates a pivotal role for admixture between foragers and farmers and/or changes in population structure during the period

between 5000 and 3500 BC in shaping the modern European gene pool.

Generally, little is known about the nature of the interactions between farmers and foragers during the Neolithic. It has been hypothesized to involve both hostile (13) and mutualistic interactions (14). There is also no real agreement on the intensity of contact; some authors claim limited or no contact, because early farmers occupied land that appears to have become devoid of Mesolithic people before their arrival (15, 16), whereas others suggest frequent contact and cooperation (17, 18).

Both hunter-gatherers and farmers used the Blätterhöhle as a burial place, and this indicates that they lived in geographically close, but distinct, niches. There may have been cultural contact between the two groups, an argument supported by the observation that—at least during the early Neolithic—flint technology traditions have a strong Mesolithic component (17, 19). The Blätterhöhle evidence is therefore consistent with a cultural contact model, whereby groups share the same or neighboring habitats, including burial places, but, nonetheless, adhere to their ancestral lifestyles and diet.

There are numerous ethnographic studies on modern hunter-gatherer communities living side

**Table 1. Results of genetic and isotope analysis, and of  $^{14}\text{C}$  dating in chronological order.** Mesolithic samples are in bold; haplotype assignments from whole mtDNA genomes are marked with an asterisk; samples falling into isotope-group 3 are highlighted in gray. Note that some samples, particularly in group 3, may have radiocarbon dates older than their true age because of the reservoir effect from freshwater fish consumption (4) (details on  $^{14}\text{C}$  data are given in table S1 and fig. S1). Cells with hyphens represent no data available.

Lab code	$^{14}\text{C}$ cal BC	mt hg	Isotopes			Isotope group
			$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	
BLA29	3020 ± 61	No U	-20.5	10.3	6.9	2
BLA28	3196 ± 103	J	-20.6	10.1	6.9	2
BLA26	3227 ± 90	-	-20.1	10.1	5.8	2
BLA5	3335 ± 136	H5	-19.8	10.3	5.9	2
BLA10*	3418 ± 63	H1c3*	-20.3	10.2	2.1	2
BLA25	3421 ± 63	U5b2a5	-19.9	10.3	6.3	2
BLA16	3429 ± 60	H11a	-20.2	10.1	4.2	2
BLA12	3449 ± 52	U5b2a2	-19.0	12.3	9.5	3
BLA1	3508 ± 102	U5b	-18.2	13.0	9.4	3
BLA13*	3513 ± 102	H5*	-20.1	10.4	5.3	2
BLA15	3571 ± 47	U5	-18.2	12.3	9.6	3
BLA21	3577 ± 43	U5	-19.0	12.2	9.3	3
BLA14*	3603 ± 49	U5b2b2*	-18.9	12.4	9.4	3
BLA24	3616 ± 56	U5	-18.7	12.5	9.5	3
BLA7*	3666 ± 20	H5*	-20.3	9.9	-0.1	2
BLA17	3681 ± 19	U5b2a2	-19.8	9.9	2.8	2
BLA9	3681 ± 19	U5b	-18.3	12.5	9.5	3
BLA8	3726 ± 38	U5	-18.8	12.3	8.7	3
BLA27	3869 ± 59	U5b2a2	-20.2	10.2	3.8	2
BLA11*	3922 ± 60	U5b2b(2)*	-18.4	12.0	9.6	3
BLA22	-	-	-19.8	10.5	4.5	2
BLA23	-	H	-20.7	10.3	5.3	2
<b>BLA20*</b>	<b>8652 ± 58</b>	<b>U5a2c3*</b>	<b>-19.2</b>	<b>8.2</b>	<b>3.9</b>	<b>1</b>
<b>BLA19</b>	<b>8638 ± 56</b>	<b>U5a</b>	<b>-19.0</b>	<b>8.0</b>	<b>1.6</b>	<b>1</b>
<b>BLA2</b>	<b>8748 ± 67</b>	<b>U/K</b>	<b>-20.0</b>	<b>7.6</b>	<b>-1.8</b>	<b>1</b>
<b>BLA6</b>	<b>8796 ± 90</b>	<b>U5b2a2</b>	-	-	-	-
<b>BLA3</b>	<b>9210 ± 29</b>	<b>U2e</b>	-	-	-	-

by side with farmers or herders (20, 21). In most cases, intergroup contact is common, e.g., the exchange of goods and food to complement their respective needs. Very often the exchange system follows a general “carbohydrates-for-protein” model, as various examples from the Philippines and Africa demonstrate [e.g. (22, 23)].

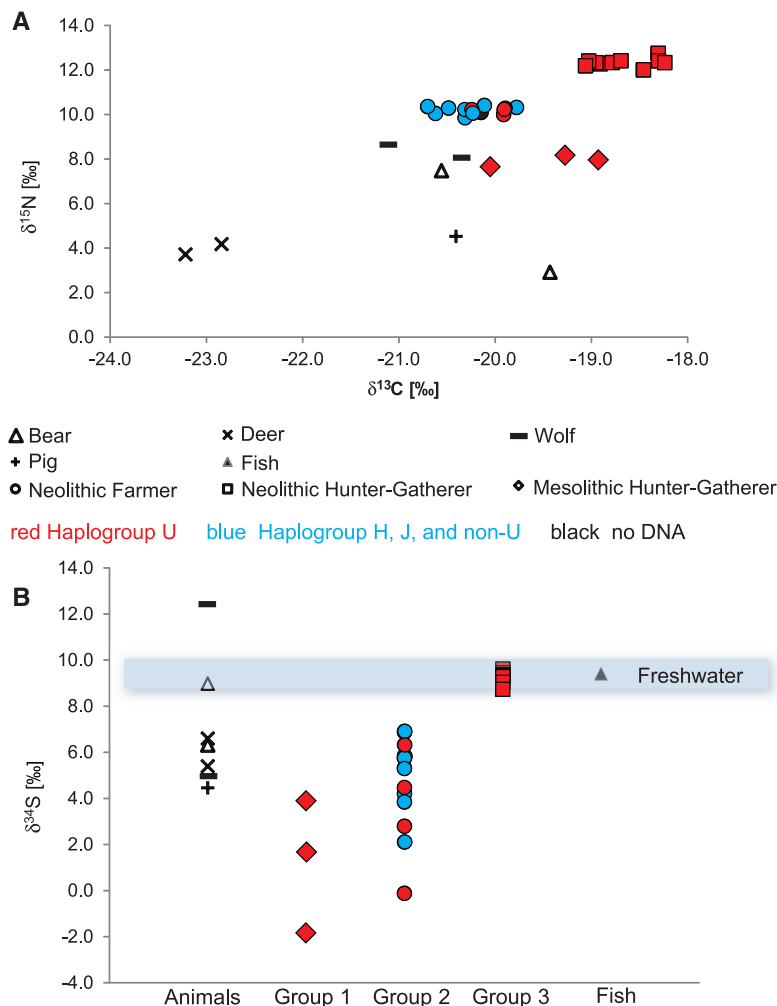
Despite these interactions, there are usually cultural norms regulating or restricting marriage between groups. Even though hunter-gatherer women can, under certain circumstances, assimilate into farmer or herder communities, this happens only rarely with hunter-gatherer men. Farming women tend not to join forager groups and mostly consider this as a social demotion (24, 25). Although our mitochondrial data only reflect the matrilineal history, these findings are consistent with the Blätterhöhle, where there is no evidence of introgression of farming females into the hunter-gatherer group; nevertheless, lineages formerly found in hunter-gatherers are also present in the group with a farming diet.

Conversely, in Northern Europe, the presence of lineages typical of farmers in the presumably

late Neolithic forager site of Ostorf (2) and in Scandinavian Pitted Ware people (26) could be interpreted as a sign of admixture in the opposite direction, i.e., women moving from farming communities to those with a hunter-gatherer lifestyle.

Undoubtedly, intermarriage between hunter-gatherers and farmers is likely to have been complex and variable across regions. Nonetheless, it is remarkable to find that a hunter-gatherer group and a group of Neolithic farmers led a parallel existence with a well-defined cultural boundary for more than 2000 years after the onset of the Neolithic in Central Europe.

It remains unclear whether the “parallel society” model we propose applies to other regions in Neolithic Europe, but the Blätterhöhle data provide the strongest evidence to date that genetically distinct hunter-gatherer groups survived for a much longer time than was previously assumed. Some of these late hunter-gatherers may have eventually converted to farming, the economy and lifestyle that became dominant for the following 5000 years.



**Fig. 2. Carbon, nitrogen, and sulfur isotope data of animals and humans from the Blätterhöhle. (A) Carbon and nitrogen. (B) Sulfur.** Samples can be divided in three clusters: group 1 (diamonds) consists of Mesolithic foragers, group 2 (circles) comprises Neolithic farmers, and group 3 (squares) represents Neolithic fisher-hunter-gatherers.

## References and Notes

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## Supplementary Material

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