Ancient DNA Yields Clues to the Puzzle of European Origins

DNA from prehistoric farmers adds fuel to a long-simmering debate over the ancestry of living Europeans; divergent male and female histories may help explain the contradictory data

In 2000, archaeologists uncovered a well-preserved male skeleton at an early farming site at Halberstadt, northwest of Leipzig, Germany. The skeleton was lying on its left side, its legs and arms tightly flexed, with three pottery bowls buried with it. The man had belonged to a central European culture called the Linearbandkeramik (LBK), characterized by large longhouses and distinctive pottery featuring sweeping striped designs. The LBK people, the first farmers known to occupy central Europe, arose in modern-day Hungary and Slovakia about 7500 years ago and within 500 years had spread as far west as France and as far east as the Ukraine.

For decades, researchers have studied the LBK culture for clues to how farming spread across Europe, an issue that is key to tracing the origins of Europe's now 700-million-strong population. The archaeological evidence shows that farming was introduced into Greece and southeast Europe from the Near East more than 8000 years ago, then spread west and north to the Atlantic Ocean. But did the farmers themselves move across Europe, in a migration of people and their genes? Or was the chief movement one of culture, as Paleolithic hunter-gatherers—whose ancestors arrived on the continent as long as 40,000 years ago—adopted farming?

Many studies over the past 2 decades have sought to test these hypotheses, often focusing on the DNA of modern Europeans in an attempt to trace their heritage. But the data have been conflicting. Now, a paper on page 1016 of this issue offers the first direct look at the DNA of early farmers themselves, including a sample from the Halberstadt skeleton. Anthropologist Joachim Burger and graduate student Wolfgang Haak of Johannes Gutenberg University in Mainz, Germany, and their colleagues found that many LBK farmers carry a mitochondrial DNA (mtDNA) type rarely found today, implying that they left little genetic legacy in living Europeans. The new data clash with some earlier studies, including Y chromosome analyses of living Europeans, which suggest that early farmers with roots in the Near East made a deep imprint on the European genome.

Dead end? This prehistoric farmer from Germany had a DNA variant that is very rare today, suggesting that his farming techniques may have spread much farther than his genes.

Because the Y chromosome is inherited through the male line, and mtDNA is passed down through women, some researchers now think that different genetic destinies of men and women could reconcile the data—and perhaps even the European origins debate. "A simple explanation for the difference is that indigenous hunter-gatherer females intermarried with [early] farmers," says Alexander Bentley, an anthropologist at the University of Durham, U.K.

The model of farming spread by migration, called demic diffusion, was formally proposed in 1984 by archaeologist Albert Ammerman and geneticist Luigi Luca Cavalli-Sforza. It postulates that large numbers of colonizing farmers spread across Europe, mating with some of the hunter-gatherers already there and displacing the rest through rapid local population growth. These growing populations then provided colonizers for still more movements west and north. Many early and some recent studies have supported the idea. For example, a widely cited 2002 paper by geneticist Lounès Chikhi of Paul Sabatier University in Toulouse, France, tracked Y chromosome variation in living Europeans and concluded that indigenous hunter-gatherers contributed less than 50% of the genes of modern Europeans; most genes, Chikhi concluded in the *Proceedings of the National Academy of Sciences*, came from the colonizing farmers.

But other researchers argued that there was little evidence that early farmers had undergone the kind of explosive population growth required by demic diffusion. Archaeologist Marek Zvelebil of the University of Sheffield, U.K., proposed an alternative model in which some colonization took place in certain areas—perhaps including the LBK region in central Europe—but that farming then spread mostly via local adoption rather than further movements of the original colonizers. A number of recent genetic studies have supported this model. For example, one study concluded that less than 25% of the mtDNA gene pool of modern Europeans could be traced to incoming early farmers (*Science*, 10 November 2000, p. 1080).

To try to get around this stalemate, Burger and Haak's team zeroed in on the mtDNA of ancient Europeans. The team tried to extract mtDNA from 57 individuals buried at 16 early farming sites, most from the LBK culture, and dated between 7000 and 7500 years ago. They succeeded with 24 of the skeletons. Moreover, the team found that six of the 24 skeletons had a mtDNA variant, called haplotype N1a, that is now very rare worldwide. Thus an apparently widespread mtDNA variant in early European farmers has left almost no trace on living

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Europeans, a finding the authors interpret as support for the cultural diffusion model.

Indeed, proponents of cultural diffusion hail the results. "It really does seem that [early farmers] must have left far fewer descendants than one might expect, given the apparent archaeological impact of the LBK at the time," says Martin Richards of the University of Leeds, U.K. Agrees Zvelebil: "This is a very important step forward. … It bypasses all the problems of extrapolation from modern DNA." But he cautions that to completely prove its case, the team should extract ancient DNA from early farmers in the Near East to see if they also have high frequencies of the N1a haplotype, as well as from hunter-gatherer skeletons in the LBK region to see if they have low frequencies.

Those who favor demic diffusion aren't yet convinced, however. "The authors are rather impatient in drawing conclusions," says Cavalli-Sforza, who thinks the results can't be properly interpreted without knowing the farmers' Y chromosome sequences too. Chikhi adds that the authors have not entirely ruled out the possibility that today's low N1a frequencies are due to chance loss of the variant. And ancient DNA pioneer Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, warns that ancient DNA studies of modern humans are notoriously unreliable because of the problem of contamination with living people's DNA. "In our experience, results from the majority of ancient human samples are irreproducible," he says.

All this leaves researchers trying to sort out the conflicting data. Most studies of mtDNA have supported the cultural diffusion hypothesis, whereas the Y chromosome data seem to favor a movement of people themselves. The idea that colonizing farmers married local hunter-gatherer women might resolve the conflict between the mtDNA and Y chromosome data and also explain the team's results, argues Bentley. "Intermarriage of farmers with indigenous women would reduce N1a in subsequent generations," he points out. Cavalli-Sforza agrees that intermarriage is a possibility, noting that men may have mated with more than one woman and that the typical LBK longhouse often had three or four hearths. "Polygynic families are a very reasonable explanation" for this architectural arrangement, he says.

For Zvelebil, the contradictory results probably indicate that neither large-scale migrations nor cultural diffusion can explain everything that happened in Europe during the adoption of agriculture. Rather, he suggests, the contribution of each of these processes probably varied from region to region: "Our prehistory was far more complicated and fascinating than either of these models allow for."

–MICHAEL BALTER

Drug Research

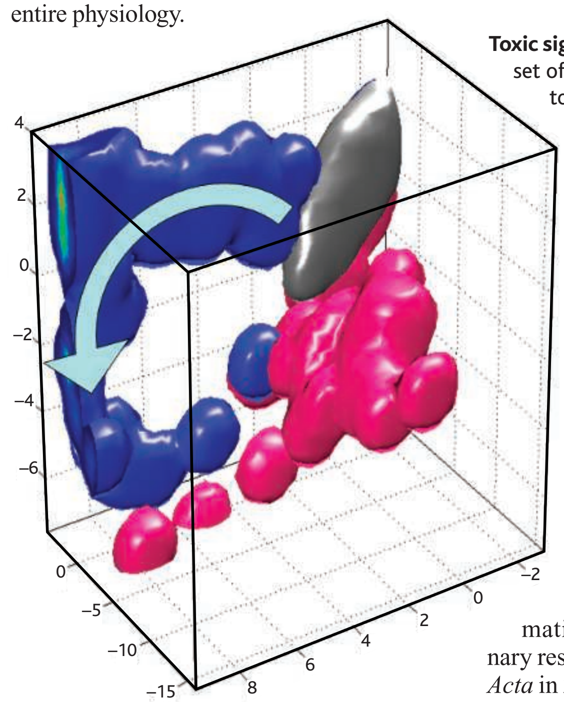
Trying to Catch Troublemakers With a Metabolic Profile

Drug discovery and toxicity research are just two areas that could benefit enormously from the use of new "metabonomic" techniques

Only one in 10 potential drug compounds ever makes it to market; the others are rejected as either too risky or ineffective. Companies have dreamed of making screening processes more efficient—and now researchers may have a way to do it. They're developing a technique based on "metabonomics," using metabolic profiles to identify toxicities rapidly and analyze the likely effects of unknown compounds. The strategy got a boost last month when several companies that had previously backed researchers at Imperial College London—including Bristol-Myers Squibb (BMS) and Pfizer—signed up to extend the work.

Metabonomics—the study of metabolic changes in urine, serum, or tissue after an organism has been exposed to a drug or other stressor—is decades old in concept. But measurement tools have become more sophisticated, making it possible to analyze data from multiple, small samples and make associations at high speed.

"It is a very powerful technology," says Bruce Carr, director of pharmaceutical candidate optimization at BMS, who has been collaborating with Imperial College researchers. Although studies suggest that companies already catch 90% of adverse effects before a drug application is submitted to the U.S. Food and Drug Administration (FDA), he believes metabolic profiling might help detect them earlier because it gives a snapshot of an organism's entire physiology.



Toxic signature. The CLOUDS program creates a set of references based on animals' responses to liver-toxic (blue) and kidney-toxic (red) compounds over time.

CREDIT: ADAPTED FROM FIGURE COURTESY OF TIM EBBELS/IMPERIAL COLLEGE

The teamwork began 5 years ago when six pharmaceutical companies including BMS and researchers at Imperial College formed the Consortium for Metabonomic Toxicology (COMET). Their goal was to develop a database of known toxins and their metabolic signatures from animal tests, to which experimental drugs with unknown toxicity could be compared. Rats were dosed at a separate facility with more than 100 toxic compounds, one per animal. The Imperial College researchers scanned urine and serum samples through nuclear magnetic resonance (NMR) spectroscopy and other technology to generate metabolic profiles of the animals.

The COMET researchers then used a computer program they had developed to assess which organs were affected. Called Classification of Unknowns by Density Superposition (CLOUDS), the software compared the NMR data—typically a hallmark signature of peaks corresponding to unknown or known metabolites—to an existing database of profiles. Tissue samples went to histology researchers for confirmation of the NMR findings. Preliminary results, published in *Analytica Chimica Acta* in 2003, demonstrated that this method

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