Time travel with the molecular clock

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Johannes Krause inside the Vindija Cave in Croatia, where the most recent fossilized bone fragments of Neanderthals in central and eastern Europe were discovered. Krause was part of the team headed by Svante Pääbo that used one of the fragments to reconstruct the first genome sequence of a Neanderthal.

Migration isn't a new phenomenon, but new insights suggest that modern-day Europeans actually have at least three ancestral populations. This finding was published by Johannes Krause and prominently featured on

the cover of *Nature*. The paleogeneticist himself is currently travellingl through time as a Founding Director of the Max Planck Institute for the Science of Human History in Jena. For him, looking back millennia into the past is no problem.

The air is abuzz with chatter and clinking glasses. It's a hot July afternoon in Tübingen and it seems like half the town is out and about today – in search of a table in the shade in front of the historic, newly restored city hall. If you were to see Johannes Krause sitting here in the café – rebellious curls, gray T-shirt, boyish appearance, with a tangible enthusiasm in his voice – you might easily mistake him for a college student in his senior year, or at most a doctoral candidate. A biologist, perhaps, or a philosopher. The indolence of college life?

That's something the 34-year-old former professor of archaeo- and paleogenetics at the Institute for Archaeological Sciences doesn't have much time for. Especially now that he has taken up the position of one of two Founding Directors of the new Max Planck Institute for the Science of Human History in Jena. At the moment, Krause is the youngest Max Planck Director.

Almost two years have passed since Johannes Krause applied for a professorship in Kiel in connection with the Max Planck Institute in Plön. "I wasn't really suited for the position, to be honest." And the twelve Max Planck Directors who interviewed him thought so, too. But then some of them asked him a question that took him by surprise: Since you're already here – could you see yourself setting up a new institute for us?

A future-oriented look into the past

What Krause didn't know was that, at that time, the Max Planck Society was already planning to realign the MPI for Economics in Jena and was

looking for a new Director. The members of the interview panel were aware of his excellent scientific career profile, which he had built up under the guidance of his mentor, Svante Pääbo, at the MPI for Evolutionary Anthropology. And so it was soon decided that he and Russell Gray – a molecular biologist from Tübingen who focuses on the analysis of ancient DNA, and an evolutionary biologist from New Zealand who conducts, among other things, linguistics research – would jointly set up the new institute. It's a future-oriented concept for looking back into the past. It's about the history of mankind. About the evolution of language and of Homo sapiens, the anatomical modern human.

They are applying their respective methods to reconstruct – or disprove – the annals of human history. After all, linguistics and genetics aren't as different as they may seem at first glance. "Migration and merging are reflected not just in genetics, but also in language," says Krause. "Russell Gray develops evolutionary trees of language families that show signs of merging as well as splitting. This allowed him to identify the time frame in which the Indo-Germanic languages diversified."

While not a single word has been passed down to us from the prehistoricage, archaeologically proven migration patterns could serve as calibration points. And since language (similar to a genome) undergoes certain "mutations" over time, models based on historical, archaeological and linguistifacts can help compute the time periods in which languages are likely to have split.

"Over the course of the past 1,000 to 2,000 years, there are several examples of populations that merged but adopted the language of the new location. By contrast, the colonization of America resulted in the language of the indigenous people becoming completely displaced," Krause explains.

The scientist from Tübingen is already enthusiastically incorporating the

research findings of his colleague from the other side of the globe into joint project ideas for the new institute. He and Gray plan to tackle many endeavors together. For example, the Migration Period that began around 375 A.D., when the Huns invaded Eastern Central Europe, and lasted until the 6th century: What really happened?

Archaeological finds from that era are typically rare and have often been subject to vague interpretations. Who fled where and merged with whom? Genes don't lie. They can provide precise data even when all that historians and archaeologists can sometimes do is hypothesize. Another example is the Austronesian expansion. Gray studies (linguistically) the settlement of Polynesia via Southeast Asia that occurred 3,000 to 4,000 years ago. "As a result of the colonization that took place over the past 500 years, many genetic patterns have been overwritten. That's why we're currently trying to reconstruct the early settlement process using very old human DNA samples taken from that region," Krause explains, outlining their first plans.

He himself is particularly interested in how modern humans spread out across Europe. "Did they come in waves because of the recurring ice ages? We don't know." A third department is also planned for the institute. "Since Gray and I have a very empirical approach to historical research, we need someone to conduct that research directly – a historian or an archaeologist." In fact, the underlying idea of the institute's guiding concept marks the renaissance of a scientific field: "More than 100 years ago, the natural sciences and the humanities drifted apart. Here in Jena, we want to try to bring them back together."

That's precisely what Krause was already doing in Tübingen. He taught classical archaeologists the foundations of genetics. To him, it comes as no surprise that this approach would work out well; after all, he spent many years of his scientific career at the Leipzig-based Max Planck Institute for Evolutionary Anthropology, which follows a similar

interdisciplinary approach.

Paleogenetics is a young scientific field that didn't came about until the 1980s. Only a small number of laboratories worldwide have the equipment and experience to analyze DNA samples taken from mummies or ancient skeletons. Even the process of merely isolating DNA from ancient remains is highly complicated, as not every shard of bone that is thousands of years old still contains usable genetic material. And if it does, the DNA is usually highly degraded and contaminated with the genetic traces of bacteria, fungi and plants that were flushed into the porous material by rainwater. Later, archaeologists and museum employees left traces of their own genetic material behind on the bone.

"Five percent of authentic human DNA – that would be considered quite a substantial amount in an ancient bone sample," Krause emphasizes. The result is usually just a tiny amount of DNA weighing no more than a few nanograms – billionths of a gram. Today, in order to prevent the researchers from leaving further traces behind on the material samples, the DNA isolation and sequencing preparations are conducted exclusively in clean-rooms by staff members wearing sterile protective clothing.

"Ancient DNA is highly degraded. The individual fragments are only about 50 base pairs long, and at the ends of these fragments, cytosine has often been chemically altered into uracil," Krause explains. "That's a shame, but it's also a sure sign that the DNA fragments being examined are, in fact, ancient genetic material." After the sample is pulverized, the protein remnants are enzymatically degraded and the remaining genetic material is isolated. The resulting DNA extract is a complex mixture containing genetic material from various organisms. The ancient human DNA is subsequently fished from that DNA soup – a process that requires a "fishing rod."

"And that often takes the form of genetic material of modern-day humans," reveals Krause, as he talks about a trick that seems simple enough. "One million single-stranded DNA fragments fixed on a small glass slide recognize their counterpart in the DNA extract from early Homo sapiens and bind to it." Now all the researchers have to do is pull the DNA that is bound to the glass slide out of the mix, decode their sequence, and analyze them using bioinformatics methods.

The Neanderthal genome, which Johannes Krause helped reconstruct, differs from that of 21st century humans by only 0.1 percent. Modern populations from around the globe have even fewer genetic differences in their DNA. A person's phenotype – the color of their skin, eyes, hair, and so on – is determined by a few dozen of our 20,000 genes. However, most of our genetic variation is shared between modern human populations. Genetically speaking, every form of racism is an absolute joke. Human populations are mostly the same.

Hitching a ride across the Atlantic

Teeth, incidentally, are the best source of ancient DNA. As a kind of "time capsule within a time capsule," they often still contain dried blood and nerve cells – and with a little bit of luck, even the genetic fingerprint of a nasty little germ. The pathogens that cause leprosy, tuberculosis and bubonic plague, for example, like to cling to nerve ends. Apart from ancient DNA and human evolution, Krause also focuses on his- torical pathogens and their co-evolution with humans.

Several years ago, he used a small number of teeth that were recovered from a London cemetery to reconstruct the genome of Yersinia pestis: the pathogen of the Black Death that wiped out nearly half of Europe's population back in the Middle Ages. What traces were left behind in human genes when our ancestors came into contact with pathogens? Along which routes and with which host did the plague or the

tuberculosis bacillus travel around the globe?

"Tuberculosis already existed on the American continent before Columbus set foot on it," Krause reports. "Using 1,000-year-old skeletal samples from Peru, we were able to show that the local pathogen is closely related to the tuberculosis pathogen that affects modern-day sea lions. It likely originated in Africa around just 5,000 years ago, and finally made its way to the indigenous peoples in South America." How was this possible? "You could say the germ 'hitchhiked' across the Atlantic on the sea lions. We assume the Native Americans in Peru contracted the disease by ingesting contaminated seal meat."

Just like humans, germs, too, undergo evolutionary changes. By comparing the genomes of ancient and modernday pathogens, scientists can determine the rate at which these germs undergo mutations. "The leprosy pathogen, for example, changes very slowly, whereas the tuberculosis pathogen changes much more rapidly. This knowledge helps us keep a better lookout for certain pathogens, because germs that change rapidly also become resistant to antibiotics more quickly." Medical experts and microbiologists hardly ever concern themselves with historical pathogens. Why is that? "They don't make use of the concept of time Krause smiles, "which means they can hardly read the 'molecular clock'."

Furthermore, ancient mutations also hold clues about the functions or structures that the pathogens used to adapt to the human host and that new drugs could potentially target. The fact that paleo-geneticists like Krause know more about the historical pathogens of the plague, syphilis, leprosy and tuberculosis than microbiologists and doctors do about their modern "descendants" is a bitter truth. All the more reason for Krause to delve deeper into this research field in Jena.

Revisiting his personal past

For Johannes Krause, making a new start in Thuringia also means returning home. He grew up in Leinefelde in the Eichsfeld region in western Thuringia, which is nestled in a hilly countryside, surrounded by people with close ties to their homeland. "I like comparing it to the village that Asterix and Obelix come from," he chuckles. This Catholic enclave in the otherwise Protestant state of Thuringia was too religious for the government of the former German Democratic Republic (GDR), which tried to counteract these sentiments in the 1960s by building his home town a socialist planned city that offered 4,000 jobs. With moderate success – "some 50 percent of the inhabitants have since moved away, because a cotton mill in central Europe doesn't make much sense."

Some of his family lived directly in the border region between the two Germanys. Visiting them meant having to file an application for a visitation permit and walking past fences and guard dogs. Johannes Krause was ten years old when the Berlin Wall came down, and the socialist state into which he had been born suddenly became little more than a controversial chapter in the country's history books.

"A good example of a social experiment that failed miserably," says Krause, who no longer has any illusions. "If it wasn't possible even in central Europe, then how is it supposed to work in other regions of the world?" He vividly remembers his first trip to the nearby town of Göttingen, in the West. The different smells wafting through the supermarket, the yoghurt shelves stocked with a seemingly endless range of flavors. He had stood in front of the toy shelves in the Karstadt department store with his mouth agape.

"Sometimes I think back to the GDR and it somehow reminds me of North Korea." Not just because of the monotonous range of available goods, but also because of the drills children had to perform in kindergarten and in school. His parents weren't able to pursue their

desired careers for having made remarks criticizing the state system. It was therefore also unlikely that Johannes Krause would have been allowed to attend university.

However, doesn't regret having grown up in a socialist state. "My political views are rather left-wing and liberal. Had I grown up in the West, I'm sure my left- wing views would be much more extreme. What I learned is that real socialism and the human factor simply aren't compatible." Yet even a child growing up in the GDR was, first and foremost, just that – a child. Like all young boys, Johannes went through a "dinosaur phase," treasuring one of the precious few books that dealt with this topic and had been published in Prague at the time.

"That was my Bible," he says, the passion still audible in his voice. "I was able to talk my father into collecting fossils with me. We went to all sorts of stone quarries in Thuringia and cracked open rocks." While he didn't come across any dinosaurs, he did find hundreds of ammonites thasoon found a new habitat in the fam- ily's garden. Shortly after the fall of the Berlin Wall, castles and ruins in the border region became his next hunt- ing grounds. "Overgrown, enchanted fairytale castles that had remained un- disturbed for decades – it was just like in an Indiana Jones movie!"

What would have become of him if the Berlin Wall hadn't come down? "I often asked myself that question. Maybe a craftsman like my father? Or a forest ranger?" He completed his community service imposed in lieu of military service at the Eichsfeld Hainich Werratal nature reserve, and really enjoyed working deep in the woods. But was that reason enough to spend the rest of his life there? No. He had been toying with the idea of becoming an archaeologist or an anthropologist for quite some time, but his career prospects were bleak. Things were to take a rather unexpected turn.

Around the year 2000 – Johannes Krause was 20 at the time – the Human Genome Project made everything and everyone believe they could soon relieve mankind from all evil. The bio-tech industry was booming, and techology was being developed at break- neck speed. "I've always been interested in the natural sciences, so why not biochemistry? I wasn't even sure what that was about, exactly, to be honest," he says today. The sheer amount of chemistry soon started to worry him, and he was all but ready to voluntarily drop out of his university in Leipzig. But then he spent a year abroad in Cork, Ireland. He was riveted by the lectures delivered by his enthusiastic genetics professor, and that was when Krause decided "to give biochemistry one more chance."

Starting out his career as an assistant in Leipzig

When he returned to Leipzig in 2003, he contacted a number of laboratories in search of a job as a student assistant. That's how he met Svante Pääbo at the MPI for Evolutionary Anthropology. The timing couldn't have been better: Pääbo had only just moved into the new institute building – there were new job openings, numerous project ideas, and lots of space to put them into practice. Krause's employment contract for his position as a student assistant stipulated a 19-hour work week, but he ended up spending almost all of his spare time at the laboratory.

First he worked on genetic studies involving chimpanzees, then cave bears. The link between genetics and archaeology – eureka! Johannes Krause had found his research field. In his thesis, he elucidated the familial relationships between mammoths and African and Asian elephants. His thesis was published in the renowned journal *Nature* – pretty cool for a junior scientist. Krause established a new method for reconstructing the entire genome sequence of ancient mitochondrial DNA.

And then Svante Pääbo offered him the opportunity to help sequence the genome of the Neanderthal .... Up until a few years before this, it had seemed downright impossible to isolate prehistoric DNA, let alone use it to reconstruct a complete genome. And the endeavor did, in fact, turn out to be extremely challenging. "In the end, we succeeded because we always bet on the right horse." The Neanderthal project was completed in 2010 with a surprising resunamely that a little bit of Neanderthal – between 2 and 3 percent – can still be found today in all people outside Africa.

This sparked a range of new projects. Krause was able to reconstruct the mitochondrial DNA extracted from a tiny knuckle bone discovered in the Denisova Cave in the Siberian Altai Mountains. He was able to show that the Denisova hominins were an independent population of the genus Homo, and that their mitochondrial DNA split away from that of the Neanderthals and modern-day humans more than one million years ago. Instead of exploring castle ruins and stone quarries near his hometown, Johannes Krause now visits excavation sites in the Middle East, Indonesia and Africa, and travels all across Europe. He also frequently finds what he's looking for in museums around the world.

Talking the curators into giving you a few hundred milligrams of mummy or skeleton bone isn't always easy. The anthropological collections in Europe in particular are a real Eldorado for the researcher – albeit an ethically questionable one, as many of the items on exhibit were looted at random from their countries of origin during the colonial era. Important artifacts were scattered across the globe or disappeared in archives, unlabeled. As a result, historical traces of entire peoples were inadvertently eradicated.

"But sometimes paleogenetics can also help tell the long-lost story of certain finds." The indigenous population of Australia was uprooted so severely over the past 200 years, for example, that the idea of genetically

reconstructing their population structure is currently under discussion.

Reviving a visionary idea

Johannes Krause is passionate about his research. He could talk for hours about further history-related questions that could be answered using genetics. And that comes as no surprise, as this young field of research is only just beginning to unfold. In Jena, he can now put his ideas into practice and stake a large "claim" for himself. The real work will begin there in early 2015. In the meantime, faster data cables are being installed in the buildings and a new laboratory will be constructed. Johannes Krause will initially be commuting back and forth between the two cities, because he will retain his professorship in Tübingen for the time being.

He has very little spare time. "Do such moments even exist?" he laughs. But that doesn't really matter. "By choosing this career, I am practically living my hobby." And this "hobby" can be combined with numerous other activities: he enjoys traveling, hiking and fishing, and tries to go jogging on a regular basis. Oh yes, and not to forget Argentine tango!

If history has taught us anything, then it is the fact that it repeats itself. Sometimes even for the better. It was 17 years ago that the Max Planck Society implemented a similarly visionary idea, which included commissioning Krause's mentor-to-be, Svante Pääbo, with setting up the MPI for Evolutionary Anthropology in Leipzig. Against the backdrop of the disastrous actions of anthropologists in the Third Reich, this decision – which came 50 years after the end of the Nazi dictatorship – was still a risky step to take. But the concept proved to be successful. Had it not been, Johannes Krause wouldn't be where he is today.

More information: Iosif Lazaridis et al. Ancient human genomes suggest three ancestral populations for present-day Europeans, *Nature*

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