on those bones and many more like them since found, seeking to discern who the Neanderthals were, how they lived, why they disappeared some 30,000 years ago, how our modern ancestors interacted with them over thousands of years of coexistence in Europe, and whether they were friend or foe, our forebears, or simply our long-lost cousins (see Figure 1.1). Tantalizing hints of behaviors familiar to us, such as care of the injured, ritualistic burial, and maybe even the production of music, emerged from archaeological sites, telling us that the Neanderthals were much more like us than is any living ape. How alike? Whether they could speak, whether they were a dead-end branch of the hominin family tree, or whether some of their genes are hidden in us today are all questions that have become an integral part of paleoanthropology, the academic discipline that can be said to have started with the discovery of those bones in Neander Valley, from which we now seemed able to extract genetic information.

Figure 1.1. A reconstructed Neanderthal skeleton (left) and a present-day human skeleton (right). Credit: Ken Mowbray, Blaine Maley, Ian Tattersall, Gary Sawyer, American Museum of Natural History.
Above, a modern reference sequence is shown. Each line below represents one cloned molecule amplified from the Neanderthal type specimen. Where these sequences are identical to the reference sequence, I have placed a dot; where they differ from the nucleotide, I have written them out. In the bottom line is the reconstructed Neanderthal nucleotide sequence. At each position, we require that a change from the reference sequence is seen in a majority of clones and in at least two independent PCR experiments (either the ones shown or others). From Matthias Krings et al., "Neandertal DNA sequences and the origin of modern humans," Cell 90, 19–30 (1997).
types of mutations are more frequent than others, and some positions in DNA sequences are more prone to mutate than others. At such positions, more than one mutation—especially the types that happen more frequently—may have occurred in the history of a DNA sequence. Therefore, to estimate the history of this particular piece of mtDNA, we needed to apply models for how we believed it had mutated and evolved, bearing in mind that certain positions might have mutated more than once, thus obscuring previous mutations. The result of such a reconstruction is depicted as a tree, in which a DNA sequence on the tip of a branch links back to a common ancestral DNA sequence. These ancestral sequences are depicted as the points where branches join on the tree (see Figure 1.3). When we did such a tree reconstruction, we saw that the mtDNAs of all humans alive today trace their ancestry back to one common mtDNA ancestor.

This finding, which was already known from Allan Wilson's work in the 1980s, is in fact expected for mtDNA, since each of us carries only a single type and cannot exchange pieces of it with other mtDNA molecules in the population. Since mtDNA is passed on only by mothers, the mtDNA

![Figure 1.3](image-url)
peered through the microscope, the appearance of the ancient tissues de­pressed me. In muscle sections, I could barely discern the fibers, let alone any traces of cell nuclei where DNA might be preserved. I was almost des­pairing, until one night I looked at a section of cartilage from a mummi­fied outer ear. In cartilage, as in bone, cells live in small holes, or lacunae, inside the compact, hard tissue. When I looked at the cartilage, I saw what appeared to be the remains of cells inside the lacunae. Excited, I stained the section for DNA. My hands were trembling as I put the slide under the microscope. Indeed, there was staining within the cellular remains in the cartilage (see Figure 2.1). There seemed to be DNA preserved inside!

With renewed energy, I went on to process all of the remaining sam­ples from Berlin. A few looked promising. In particular, the skin from the left leg of the mummy of a child showed what were clearly cell nuclei. When I stained a section of the skin for DNA, the cell nuclei lit up. Since this DNA was in the cell nuclei, where the cellular DNA is stored, it could not possibly be from bacteria or fungi because such DNA would appear at random in the tissue where the bacteria or fungi were growing. This was unambiguous evidence that DNA from the child herself was preserved. I took many photos through the microscope.

I found three mummies with staining of the cell nuclei showing the presence of DNA. The child seemed to have the largest number of

![Figure 2.1](image-url). Microscopic picture of cartilage tissue from an Egyptian mummy from Berlin. In some lacunae, cell remains light up suggesting that DNA may be preserved. Photo: S. Pääbo, Uppsala University.
was the first-ever peek back in time at the genes of the ancestor populations of animals living today. We published our findings in the *Journal of Molecular Evolution* and were pleased to find a glowing comment about our work in *Nature* by up-and-coming evolutionary biologist Jared Diamond, who said that the new techniques made possible by the PCR meant that "old specimens constitute a vast, irreplaceable source of material for directly determining historical changes in gene frequencies, which are among the most important data in evolutionary biology." He also said that "this demonstration project will make life harder for those who are too narrow-minded to understand the scientific value of museum specimens."

However, to me, human evolutionary history was the Holy Grail, and I wondered whether the PCR could open a window into our own past. In Uppsala, I had gotten a sample from some gruesome yet amazing discoveries made in Florida sinkholes. In these water-filled alkaline deposits, ancient Native American skeletons were found; inside the crania, the brains, although slightly shrunken, were preserved in surprising detail. Using old-fashioned techniques, I had shown that the sample contained preserved human DNA, and I presented these results at Cold Spring Harbor,
conclusions from my awareness of the PCR’s extreme sensitivity. At Berkeley, and during the first period in Munich, we would extract DNA from museum specimens on our lab benches—the same benches where we handled large amounts of DNA from humans and other organisms we were interested in. If even a microscopic droplet of a modern DNA solution made it into the ancient DNA extract, the modern DNA would overwhelm the few ancient molecules that might have come from the ancient tissue. This could well happen even if we made no obvious mistakes, such as forgetting to change the plastic tip of a pipette.

It became clear to me that what we needed was to achieve complete physical separation of the extraction and handling of DNA from ancient
happened independently more than once—if history repeated itself, so to speak—it suggests that there are a limited number of ways in which animals can adapt to an ecological challenge. Each such case of convergence, when two or more unrelated organisms independently evolve similar behaviors or body shapes, is evidence that evolution follows rules—and is helpful in deducing how these rules work. An example of this was the marsupial wolf that we had studied in Zurich and Berkeley. In the case of the tree sloths, just as in the case of the marsupial wolf, we could determine whether convergence had occurred if we could clarify how Darwin's extinct giant ground sloth was related to the two-toed and three-toed tree sloths.

I visited the Natural History Museum in London and spent some time there with the amiable curator of Quaternary mammals, Andrew Currant, an expert on mammal paleontology with a build not unlike that of a large Pleistocene mammal. He showed me some of the fossilized bones that Darwin had brought back, and he allowed me to cut a small piece from two of the Patagonian Mylodon bones in their collection. I also visited the American Museum of Natural History, in New York, and got samples for our study there. But it was in Andrew’s museum that I experienced a vivid demonstration of how readily the ancient animal specimens we studied might become contaminated. As I was examining sloth bones with Andrew, I asked him if they had perhaps been treated with varnish. To my amazement, he picked up a bone and licked it. “No,” he said, “these have not been treated,”
explaining that if a bone had been treated with varnish, it would not absorb saliva. In contrast, an untreated bone would do this so efficiently that one’s tongue tended to adhere to the bone. I was horrified and wondered how many times this “test” had been done during the hundred years or more that some of the bones we worked with had been in museums.

Once the samples were back in Munich, Matthias Höss applied his skills to them. As always, I insisted that we first pay attention to the technical side of things. My interest in sloths was after all driven mainly by an interest in how to retrieve ancient DNA. Matthias used a rough assay to estimate the total amount of DNA in his Mylodon extract and another crude assay to measure how much of that was similar to modern sloth DNA. It turned out that about 0.1 percent of the DNA in our best Mylodon bone extract was from the animal itself, the rest having come from other organisms that had lived in the bones after the giant sloth died. This has turned out to be typical of many ancient remains we have since studied.

Focusing on mitochondrial DNA fragments, Matthias managed to use the PCR to reconstruct a stretch of Mylodon mtDNA more than a thousand nucleotides long by amplifying short overlapping pieces. By determining and comparing the same sequences from samples from living sloths, he could show that the giant ground sloth, which stood ten feet tall on its hind legs, was more closely related to the present-day two-toed tree sloth than to the three-toed tree sloth. This was important, since if the two- and three-toed sloths had been most closely related to each other and more distantly related to Mylodon (which was the opinion of most scientists at that time), it would have suggested that they had a common ancestor who became tree-dwelling. Our result suggested that sloths had at least twice evolved into forms that were small and spent most of their lives in trees (see Figure 5.2).

![Figure 5.2](image)

_Figure 5.2. A tree showing that the Mylodon is more closely related to the two-toed than to the three-toed sloth, suggesting that sloths started to live in the trees twice during their history. From Matthias Höss et al., “Molecular phylogeny of the extinct ground sloth Mylodon darwini,” Proceedings of the National Academy of Sciences USA 93, 181–185 (1996).
from a part where the shaft had no ridges or other features of interest to paleontologists, who study how muscles had attached to the bone. It also became clear that we would not be allowed to remove the sample ourselves. Ralf and a colleague came to see us in Munich, and we gave them a sterile saw, protective clothing, sterile gloves, and containers in which to store the sample—and off they went. In the end, it was probably fortunate that I was not allowed to put the saw to the archetypal Neanderthal myself. I would probably have been too intimidated by this iconic fossil and would have cut off a very small piece, perhaps too small for success. When we received the sample, we were impressed by the size of what they had removed—3.5 grams of what looked like very well-preserved whitish bone (see Figure 5.3). Ralf reported that when they sawed through the bone, a distinct smell of burnt bone spread through the room. This, we believed, was a good sign; it had to mean that collagen, the protein that makes up the matrix of bone, had been preserved. It was with awe and trepidation that I approached my graduate student Matthias Kring, who had spent more than a year on fruitless attempts to extract DNA from Egyptian mummies—with the plastic bags containing the piece of the Neanderthal type specimen and asked him to apply our latest and best methods to it.
found bone fragments of several Neanderthals but no spectacular crania like those found in Krapina. Malez also found enormous amounts of cave-bear bones. His finds are housed in Zagreb, too, in the Institute for Quaternary Paleontology and Geology, which belongs to the Croatian Academy of Sciences and Arts. I arranged to visit both this institute and the Museum of Natural History. In August 1999, I arrived in Zagreb.

The Krapina Neanderthal collection was extremely impressive, but I was skeptical about its potential for DNA research. The bones were at least 120,000 years old and therefore older than anything we had found to yield DNA. The Vindija collection looked more promising. First of all, it was younger. Several layers in the excavation had yielded Neanderthal remains, but the uppermost and thus the youngest one to do so was between 30,000 or 40,000 years old—young, as far as Neanderthals go. I saw a second exciting feature of the Vindija collection: it was full to overflowing with ancient cave-bear bones. They were stored, according to bone type and layer, in innumerable paper sacks that were coming apart in the humidity of the Quaternary Institute's basement. There were sacks full of ribs, others full of vertebrae, others of long bones, and yet others of foot bones. It was an ancient DNA gold mine.

In charge of the Vindija collection was Maja Paunovic, a woman of a certain age who spent her days in an institute without public exhibitions

**Figure 6.1. Vindija Cave in Croatia. Photo: J. Krause, MPI-EVA.**
But most importantly I could for the first time design a clean room for ancient DNA extractions that was to my specifications. This largely meant giving free reign to my paranoia about contamination from human DNA stuck to dust particles. The “clean room” was in fact not just a single room but several rooms. They would be located in the basement of the building, where you could enter the clean facility without coming even close to laboratories where modern DNA was being handled. In the clean facility, you would first enter a room where you would change to sterile clothing. You would then enter a preliminary room where somewhat “dirty” work, such as grinding bone samples to powder, would be done. From there you would enter the innermost room, where DNA extractions and manipulations of the extracted DNA would be performed. Here, too, the valuable DNA extracts would be stored in special freezers. All work here would be done in hoods where the air was filtered (see Figure 7.1). In addition, the air of the entire facility would be circulated and filtered. It was to be sucked through a grid on the floor, and 99.995 percent of all particulates larger than 0.2 thousands of a millimeter would be removed from it before it was returned to the room. We constructed not one but two such facilities in the basement so that different types of work—for example, on extinct animals and on Neanderthals—could be separated. No reagents or equipment would ever be allowed to pass from one of the clean rooms to the other, so that if we ever

Figure 7.1. The innermost of our clean rooms at the Max Planck institute in Leipzig. Photo: MPI-EVA.
agriculture. In short, we would miss much of the world’s genetic diversity. Second, we could collect people according to land area, taking a sample every couple of square miles. But this, in addition to posing formidable logistic challenges, would result in over-sampling of sparsely populated areas such as the Arctic. The third option, which we finally adopted, was to focus on major language groups. We argued that major language groups (such as Indo-European, Finno-Ugric, and so on) reflect some approximation of cultural diversity going back more than 10,000 years. So by focusing on samples representative of major language groups, we could increase our chances of sampling most groups that have had long, independent histories. We would therefore hopefully cover more of human genetic variation.

Fortunately, others had come up with this idea before us so we were able to rely upon DNA samples collected by the distinguished Italian geneticist Luca Cavalli-Sforza at Stanford University. From those samples, Henrik selected sixty-nine men representing all major language groups and sequenced the 10,000 nucleotides in each of them. When he compared the DNA sequences in randomly chosen pairs of men, he found an average of just 3.7 nucleotide differences. Just as had been seen for the mtDNA, he found more variation between pairs of individuals from within Africa than from outside Africa. To gain some perspective on these results, he next turned to the closest living relatives of humans: the chimpanzees.

![Figure 8.1. Tree of humans and great apes indicating approximate times when they may have shared common ancestors (although these dates are very uncertain). Modified from Henrik Kaessmann and Svante Pääbo “The genetical history of humans and the great apes,” Journal of Internal Medicine 251: 1-18 (2002).]
amplified pieces of two more single-copy genes: one encoding a protein regulating the release of neurotransmitters in the brain and one encoding a protein that binds vitamin A and is produced by the rods and cones in the eyes. He was successful in both cases.

Since we had struggled so long to retrieve nuclear DNA, Alex’s mammoth results were very welcome indeed and for several days I was very happy about them. But of course I wasn’t all that interested in mammoths. I was interested in Neanderthals, and I was painfully aware that there were no Neanderthals in permafrost. I urged Alex to go back and try the cave-bear remains from Vindija again, to see if he could retrieve nuclear DNA from remains that were not frozen. He analyzed mitochondrial DNA from several Croatian cave bears and identified one bone that seemed to contain
that the excavator Mirko Malez had thought were from cave bears but that Tim thought could potentially stem from Neanderthals.

Looking at these bone fragments, I was reminded of something Tim had mentioned to me when we met at Berkeley a year earlier. The Vindija Neanderthal bones—all of them—were crushed into small fragments. This is typical of many, even most, sites where Neanderthal bones are found. Of course, it is not surprising that bones thousands of years old are not in good condition. But there are often cut marks on the bones where muscles and tendons had been attached as well as cut marks on the skulls. In short, the skeletons had clearly been deliberately de-fleshed, and bones containing marrow had been crushed, presumably to get to their nutritious contents. Tim had pointed out to me the similarity of this pattern of Neanderthal bone fragmentation to a gruesome Anasazi site from the American Southwest, where around AD 1100 some thirty men, women, and children had been butchered and cooked. He told me that the way in which many Neanderthal bones were crushed was similar to the way the bones of animals, such as deer, that were butchered by Neanderthals were crushed (see Figure 12.1). We will probably never know how common it was for Neanderthals to kill and eat other Neanderthals, or, indeed, whether these Neanderthal corpses might have been butchered and perhaps eaten as part of some mortuary ritual. But given that Neanderthal skeletons are found

![Figure 12.1. The bone 33.16 from Vindija Cave that we used for sequencing the Neanderthal genome. It has been crushed, presumably to get to the nutritious marrow. Photo: Christine Verna, MPI-EVA.](image)
for Quaternary Paleontology and Geology making a preliminary catalog of all the Neanderthal bones in the Vindija collection. Johannes and I spent four days in Zagreb and then returned to Leipzig in the company of Pavao, Željko, and Johnny, who carried eight bones from Vindija in sterile bags, including the celebrated Vi-80, now officially known as Vi-33.16 (see Fig. 12.1).

We arrived late at night. The first thing we did next morning was to bring the bones to the Department of Human Evolution, where, still in their bags, they were scanned by computer tomography so that their morphology would be forever preserved in a digital form. Then the bones went into the clean room, and Johannes took over.

Using a dental drill with a sterilized bit, he removed two or three square millimeters of the surface from each bone. Then he drilled a small hole into the compact part of each bone, pausing frequently to avoid heating the bone and potentially damaging the DNA (see Figure 12.3). He collected about 0.2 grams of bone, adding it to a solution that within a few
hours bound the bone’s calcium. What was then left of the bone was a pellet of proteins and other components from its nonmineral portion. The DNA, however, was in the dissolved liquid part, and Johannes purified it by letting it bind to silica—the technique that Matthias Höss, fourteen years earlier, had found to be particularly good at isolating DNA from ancient bones.

To make the DNA molecules amenable to 454 sequencing, Johannes used enzymes to fill in and chew away any unraveled single-stranded DNA at the ends of molecules. That enabled him to use a second enzyme to fuse short synthetic pieces of modern DNA, called adaptors, to the ends of the ancient DNA. After adaptors have been added to DNA molecules, they can be “read” by sequencing machines just like books, so the collection of them is called a library. The adaptors had been synthesized especially for this project, and they contained a short additional sequence of four bases, TGAC, positioned so that it would abut the ancient fragments as a kind of marker or tag. This was one of those small technical details that often make a huge difference in molecular biology in general and ancient DNA research in particular. We had introduced these tags because our ancient DNA library had to leave the clean room to be sequenced on the 454 machine. In order to ensure that DNA from other libraries in our
a project, weighing individual abilities in this regard. As the Neanderthal crisis loomed over the group, however, I was amazed to see how readily the self-centered dynamic gave way to a more group-centered one. The group was functioning as a unit, with everyone eagerly volunteering for thankless and laborious chores that would advance the project regardless of whether such chores would bring any personal glory. There was a strong sense of common purpose in what all felt was a historic endeavor. I felt we had the perfect team (see Figure 13.1). In my more sentimental moments, I felt a love for each and every person around the table. This made the feeling that we'd achieved no progress all the more bitter.

During the spring of 2007, the Friday meetings continued to show our cohesive group from its best side. People threw out one crazy idea after another for increasing the proportion of Neanderthal DNA or finding microscopic pockets in the bones where preservation might be better. It was almost impossible to say who came up with which idea, because the ideas were generated in real-time, during continuous discussions to which everyone contributed. We started talking about ways to separate the bacterial DNA in our extracts from the endogenous Neanderthal DNA: maybe the bacterial DNA differed from the Neanderthal DNA in some feature that we
X and Y. Because females carry two X chromosomes while males carry one X chromosome and one Y chromosome, if a bone came from a female, we should find only X chromosome fragments and no Y chromosome fragments. Therefore, any Y chromosome fragments we detected in libraries derived from a female’s bone would be indicative of contamination by modern males.

This analysis, suggested during one of our Friday meetings in Leipzig, initially sounded simple. But as with so many of the things Ed did, it was not as straightforward as it seemed. The complication was that, although the X and Y chromosomes are morphologically distinct, some of their parts share a close evolutionary relationship. The DNA they share as a result of this relationship could confuse the analysis when we mapped our short DNA fragments. To avoid this problem, Ed identified 111,132 nucleotides on the Y chromosome that weren’t similar to anything else in the genome, even if these bits were fragmented into pieces as small as 30 nucleotides in length. When he looked among the Neanderthal DNA fragments, he found just four fragments carrying these Y chromosomal sequences; if the bones we used had all come from males, he would have expected to see 666 of them.
genetic contribution only in Europe, where Neanderthals had lived. We saw it also in China and Papua New Guinea. How could this be? Absent-mindedly, I started to clean my desk. Slowly at first but then with increasing energy, I tossed out debris from years-old projects. Dust whirled into the air from layers deep on my desk. I needed to start a new chapter. I needed a clean desk.

Doing domestic tasks sometimes helps me think and as I cleaned, I visualized modern humans as arrows on a map coming out of Africa and meeting Neanderthals in Europe. I could imagine them having babies with Neanderthals—babies who then became incorporated among the modern humans, but I struggled to see how their DNA came to East Asia. It was possible that subsequent migration among modern humans might have brought Neanderthal DNA to China, but it seemed we would then find less similarity on average between a Chinese person and Neanderthals than between a European person and Neanderthals. Then it dawned on me: my imaginary arrows showing modern humans coming out of Africa passed

![Diagram of human migrations](image)

**Figure 18.1.** An illustration of the idea that if the Neanderthals mixed with early modern humans leaving Africa, and these went on to populate the rest of the world outside Africa, they would carry Neanderthal DNA with them to regions where Neanderthals never existed. For example, about 2 percent of the DNA of people even in China comes from Neanderthals. Photo: Pääbo, MPI-EVA.
from, but we extracted DNA from them and showed that they contained Neanderthal mtDNA. Together with Anatoly, we then published a paper in Nature in 2007 that extended the range where Neanderthals had lived by at least 2,000 kilometers further east of what had been commonly believed. Prior to our paper, no Neanderthal had been confirmed east of Uzbekistan.

In the spring of 2009 we received another bone fragment from Anatoly. His team had discovered that fragment during the previous year in Denisova Cave, another cave in the Altai region located in a valley that connects the Siberian steppes in the north to China and Mongolia in the south. The bone was minuscule, and I hadn't attached very much importance to it, thinking only that we would see whether it contained any DNA at some point in the future when there was time. Perhaps it would prove to be Neanderthal, which would enable us to gauge the extent of mtDNA variation among the easternmost Neanderthals.

Johannes had now found the time to extract DNA from the bone; and Qiaomei Fu, a talented young graduate student from China, had made a library and used a method that Adrian Briggs, the British graduate student in our lab, had developed to fish out mtDNA fragments from the library. They found a very large amount of mtDNA—in total, 30,443 fragments, which enabled them to assemble the complete mitochondrial genome with
morning walking along the windy beach below Cold Spring Harbor and thought about the young person who had died far away in a Siberian cave many thousands of years ago. All that remained of that life was a tiny speck of bone, but it was enough to tell us that she represented something unknown to us, a group of humans who had left Africa before the ancestors of the Neanderthals but after *Homo erectus*. Could we find out what this group was?

When I got back to Leipzig I sat down with Johannes and the others to discuss our next steps. The analyses of the Neanderthal genome were drawing to a close, so people had time on their hands to think about these startling findings. The first thought was whether there could be something wrong with the DNA sequence Johannes had reconstructed. Qiaomei and Johannes had retrieved thousands of mtDNA fragments and much less than 1 percent of them carried substitutions that were suggestive of contamination. Since the mtDNA looked quite different from present-day human mtDNA, it couldn't be contamination from anyone today. Earlier in my career I had often worried about fragments of mtDNA that thousands or millions of years in the past had become integrated on a cell's nuclear
would we submit it? The editors at Science were already impatiently waiting for our Neanderthal genome paper. To approach them about a different paper on a different topic might make them write us off as unable to finish one project, let alone two. So we decided to contact Nature. During a long layover at the airport in Moscow, I wrote an e-mail to Henry Gee, the senior editor who handles paleontology at Nature, and to Magdalena Skipper, the editor who handles genomics. I told them that we had a paper almost finished that described “what we interpret as a new hominin species based on a complete mitochondrial DNA sequence that diverged from the human line about twice as long ago as the Neanderthal mtDNA.” I was all too aware that the publication process could drag on for many months. It could even end in rejection, after months of dithering with reviewers and editors, after which we would need to submit to another journal and endure another similarly lengthy process. I didn’t want that to happen this time so I told them that we had direct competition and would be grateful if the paper could be handled quickly. An hour and fifteen minutes later, Henry Gee replied with “How exciting! Prediction is very hard, especially about the future. However, when you send it in, we’ll give it topmost priority.”

As soon as we were back in Leipzig we finished the manuscript, which we entitled “The Complete mtDNA Genome of an Unknown Hominin from Neanderthal Times.”
mapping was preliminary. In spite of this, we distributed the data to the X-Woman Consortium. Not long after we had submitted the final version of the revised mtDNA paper to *Nature*, Nick Patterson sent me a report on its preliminary analysis of Udo’s preliminary mappings. When I read it, I felt grateful to the reviewer who had convinced us not to name a new species. Nick had found two things.

First, he found that the nuclear genome of the Denisova finger bone was more closely related to the Neanderthal genome than to the genomes of people living today. In fact, it seemed to be only slightly more different from the Neanderthal genome than the deepest differences one could find among humans living today—for example, between the Papua New Guinean individual we had sequenced and the African San individual. This was quite a different picture than the one painted by the mtDNA results alone, and my immediate suspicion was that gene flow from some other more ancient hominin in Asia was responsible for introducing the mtDNA into the Denisova individuals. After all, we had just shown that modern humans had interbred with Neanderthals, so gene flow seemed a reasonable guess. But it was something we needed to think carefully about.