



Close Encounters Of the Prehistoric Kind

The long-awaited sequence of the Neandertal genome suggests that modern humans and Neandertals interbred tens of thousands of years ago, perhaps in the Middle East

IT'S THE MYSTERY OF MOUNT CARMEL. ON this limestone ridge overlooking the coast of Israel, modern humans lived in caves off and on for tens of thousands of years, starting more than 100,000 years ago. Then, perhaps as early as 80,000 years ago, members of another species reached and occupied the caves: heavy-bodied Neandertals, who were escaping a cold spell in Europe and moving south into the Middle East. Did the two species meet here? Did they mate?

The archaeological record in the caves is ambiguous on that question, and anthropologists have fought bitterly over it. Some claim that the anatomy of fossils shows that Neandertals, our closest cousins, did mate with modern humans, either in the Middle East or in Europe. But others thought modern humans coming out of Africa completely replaced Neandertals with little or no interbreeding. And the genetic evidence from ancient bones showed no sign that Neandertals had swapped genes with our ancestors—until now.

On page 710, an international team of researchers presents their first detailed analysis of the draft sequence of the Neandertal genome, which now includes more than 3 billion nucleotides collected from the bones of three female Neandertals who lived in Croatia more than 38,000 years ago. By comparing this composite Neandertal genome with the complete genomes of five living humans from different parts of the world, the researchers found that both Europeans and Asians share 1% to 4% of their nuclear DNA with Neandertals. But Africans do not. This suggests that early modern humans interbred with Neandertals after moderns left Africa, but before they spread into Asia and Europe. The evidence showing interbreeding is “incontrovertible,” says paleoanthropologist John Hawks of the University of Wisconsin, Madison, who was not involved in the work. “There’s no other way you can explain this.”

Kissing cousins. A few Neandertals mated with early modern humans and passed on some of their genes to living humans.

As a result, many people living outside Africa have inherited a small but significant amount of DNA from these extinct humans. “In a sense, the Neandertals are then not altogether extinct,” says lead author Svante Pääbo, a paleogeneticist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, who was surprised to find he was part Neandertal. “They live on in some of us.”

The team also used the Neandertal DNA like a probe to find the genes that make us modern. Even though the genomes of humans and Neandertals are 99.84% identical, the researchers identified regions that have changed or evolved since our ancestors and Neandertals diverged sometime between 270,000 and 440,000 years ago—their new, slightly younger estimate of the split. So far, the team has detected tantalizing differences in genes involved in metabolism, skin, the skeleton, and the development of cognition, although no one knows yet how these genetic changes affect physiology. “This is a groundbreaking study!” enthuses evolutionary geneticist Hendrik Poinar of McMaster University in Hamilton, Canada. “We can actually discuss an extinct human species—Neandertals—on a genetic level rather than strictly on morphological grounds.”

Mixed marriage

The discovery of interbreeding in the nuclear genome surprised the team members. Neandertals did coexist with modern humans in Europe from 30,000 to 45,000 years ago, and perhaps in the Middle East as early as 80,000 years ago (see map, p. 681). But there was no sign of admixture in the complete Neandertal mitochondrial (mtDNA) genome or in earlier studies

of other gene lineages (*Science*, 13 February 2009, p. 866). And many researchers had decided that there was no interbreeding that led to viable offspring. “We started with a very strong bias against mixture,” says co-author David Reich of Harvard Medical

School in Boston. Indeed, when Pääbo first learned that the Neandertal DNA tended to be more similar to European DNA than to African DNA, he thought, “Ah, it’s probably just a statistical fluke.” When the link persisted, he thought it was a bias in the data. So the researchers used different methods in different labs to confirm the result. “I feel

Online
sciencemag.org
S Special online feature and podcast on the Neandertal genome.

confident now because three different ways of analyzing the data all come to this conclusion of admixture,” says Pääbo.

The finding of interbreeding refutes the narrowest form of a long-standing model that predicts that all living humans can trace their ancestry back to a small African population that expanded and completely replaced archaic human species without any interbreeding. “It’s not a pure Out-of-Africa replacement model—2% interbreeding is not trivial,” says paleoanthropologist Chris Stringer of the Natural History Museum in London, one of the chief architects of a similar model. But it’s not wholesale mixing, either: “This isn’t like trading wives from cave to cave; the amount of admixture is tiny,” says molecular anthropologist Todd Disotell of New York University in New York City. “It’s replacement with leakage.”

Although the 1.3-fold coverage of the Neandertal genome is a remarkable technical feat, one-third of the genome is still murky. In a separate paper (p. 723), the team describes and successfully tests a new method for filling in gaps in the rough draft of the genome.

The team also used three methods to nail down the interbreeding result. First, they compiled the Neandertal genome using DNA from the limb bones of three female Neandertals who lived in Vindija Cave in Croatia from 38,000 to 44,000 years ago; they confirmed parts of the genome with much smaller amounts of DNA from Neandertals who lived in Spain, Germany, and Russia.

Once they were satisfied that the composite genome was a fair representation of Neandertals from across a great part of their geographical range, researchers compared the Neandertal genome to a chimpanzee’s to determine which genetic variants were primitive, ancestral forms. Then they compared the new, derived genetic variants in Neandertals to those in the complete genomes of five living humans, including a San from Southern Africa, a Yoruba from West Africa, a Papua New Guinean, one Han Chinese, and one French European.

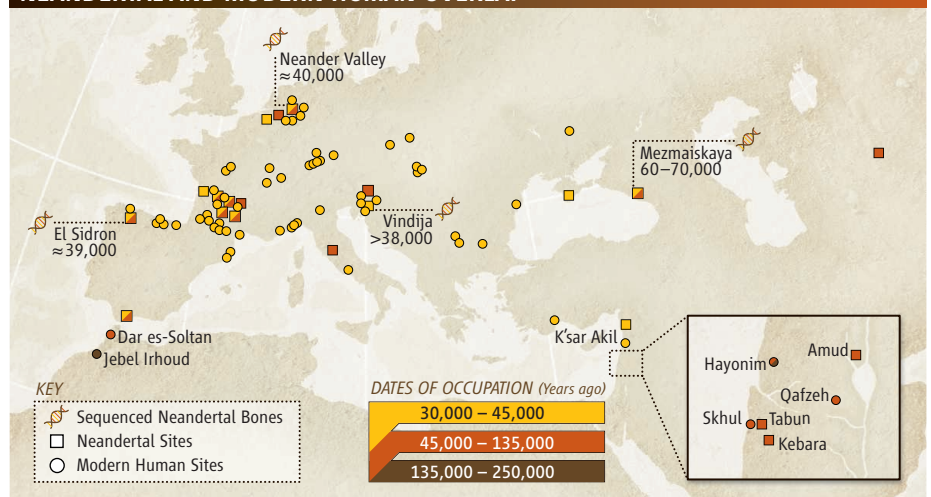
The team measured the genetic proximity of Neandertals to pairs of modern humans from different continents, first using single-nucleotide polymorphisms (SNPs), or sites in the genome where a single nucleotide differs between individuals. When they compared a Neandertal with a European and an Asian, they found that the Neandertal always shared the same amount of derived (or more recently evolved) SNPs with each of them. But when they compared a Neandertal with an African and a European, or with an African and an Asian, the Neandertal always shared



Clean cave. Researchers carefully worked with DNA from three Neandertals’ bones (left) in Vindija Cave, Croatia, where the fossils were found (right).



NEANDERTAL AND MODERN HUMAN OVERLAP



Points of contact. Archaeological data suggest that Neandertals and early modern humans may have overlapped early in the Middle East and later in Europe.

more SNPs with the European or Asian than with the African. “We’ve shown that Neandertals are significantly more closely related to non-Africans than Africans on average,” says Reich.

Even though they looked at just two Africans for this part of the study, those two have a particularly ancient, diverse heritage, so they are a good proxy for much of the genetic diversity in Africa. But sequencing additional Africans would be a good idea, says Reich.

For now, it seems Neandertals interbred with the ancestors of Europeans and Asians, but not with the ancestors of Africans. At first, “we were baffled that this affinity with

Neandertals was not only in Europe and West Asia [where it was most expected], but also in Papua New Guinea” where Neandertals never set foot, says Pääbo.

To be certain, they used two other methods to detect gene flow between Neandertals and Eurasians. Using the published genome of an African American from the Human Genome Project, they compared large regions of African and European ancestry in this single genome to Neandertal regions. In this person’s genome, the European and Neandertal segments were more similar to each other than either was to the African segments.



Cloned Neandertals Still in the Realm of Sci-Fi

Science-fiction writers have been resurrecting Neandertals in novels for decades, imagining what it would be like to see and communicate (not to mention mate) with another species of human. So once the idea of sequencing the Neanderthal genome became more than a glimmer in a paleogeneticist's eye, some have asked, "Could we, should we, would we, bring this extinct human species back to life?" After all, biologists are trying to bring back the woolly mammoth by cloning. But for both technical and ethical reasons, experts say, bringing back a Neanderthal is a pipe dream.

Could we do it? Robert Lanza laughed at the thought. Chief scientific officer for Advanced Cell Technology in Worcester, Massachusetts, he and his colleagues have cloned species from

cows to goats to mice and extended their efforts to include endangered species and human embryos. But cloning Neandertals is fantasy, says Lanza. "You can't clone from stone, and you can't clone from DNA that has been destroyed from weather and the elements," he points out.

The Neanderthal genome sequence reported on page 710 (and see main text, p. 680) reflects the battered state of the starting DNA, which came from bones that are 38,000 to 44,000 years old. Because the isolated DNA was in pieces typically about 50 bases long, there are many missing stretches, particularly repetitive regions. "We will never have a finished sequence for the Neanderthal the way we have for a mouse," says Svante Pääbo, who led the Neanderthal sequencing project at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. *Jurassic Park* aside, recon-

structing an organism with a partial genome would be like constructing a building with a partial blueprint.

Even if scientists had the complete genome, it wouldn't be enough. DNA itself doesn't tell the whole story. Chemical modifications to the genome, the way chromosomes arrange in the nucleus, and maternal components in the egg all play a role in translating a genetic blueprint into a viable individual. "It's not just the DNA; there's a lot else going on," says Lanza. None of that information is even available for Neandertals.

Then, too, cloning doesn't typically start with a genome; it starts with two cells. One cell provides a nucleus (with DNA inside), and one is an egg cell, most often of the same species, whose DNA has been removed. The nucleus is then transferred to the egg, sometimes by fusing the two cells. "If you have just got DNA, you are asking an enormous amount of the oocyte that you are going to put the DNA into," explains Ian Wilmut, who cloned Dolly the sheep and now works at the University of Edinburgh in the United Kingdom. "It has to reform the nucleus and reprogram [the DNA]."

That leads to the next problem: What species' egg would play host to this DNA? The obvious candidate would be a modern human egg, but they are notoriously fickle and don't take well to nuclear transfer, even of modern human DNA. "There's something different about primates that we haven't identified," says Wilmut. "[Cloning] works very poorly." And incompatibilities between Neanderthal DNA and the human egg might further diminish the chances of a viable embryo.

Molecular geneticist George Church of Harvard University has proposed another approach: modify the DNA in a human cell line to resemble the Neanderthal. "This is a daunting task, but with future technological developments and enough time and money, it may be possible," says Adrian Briggs, who worked on the

Finally, population geneticist Rasmus Nielsen of the University of California (UC), Berkeley, scanned the human genome for "ancient" genomic segments—those that might predate the time when modern humans arose, about 200,000 years ago. Before receiving the Neanderthal DNA sequences, he identified 13 genomic regions that were unusually variable, and therefore likely to be evolutionarily ancient, in 48 people outside of Africa. He identified 13 "old" variants as possibly coming from Neandertals or other archaic ancestors, because they were missing from the genomes of 23 African Americans (used as proxies for Africans). Then the team looked

in the Neanderthal genome—and found 10 of the 13 ancient variants. "There are places in the genome where we can say this section is really, really likely to be from a Neanderthal," says Reich.

When and where did modern humans pick up those Neanderthal genes? The most likely scenario "was the movement of a few Neandertals into a group of moderns," says co-author and population geneticist Montgomery Slatkin of UC Berkeley. If a few Neandertals interbred with members of a small population of modern humans, Neanderthal gene variants might persist in subsequent generations of modern humans if the interbred population expanded rapidly,

thereby spreading Neanderthal DNA widely.

This scenario apparently fits with fossils and stone tool data from the Israeli caves such as Skhul, Qafzeh, and Tabun, where Neandertals show up in the region as early as 80,000 years ago, when moderns were already there. Although each group may have occupied the caves intermittently, some say they may have overlapped for up to 10,000 years. Neandertals and moderns apparently even occupied the same cave, Tabun, at different times. The two species had much in common: Both lived in caves, used similar toolkits (although Neandertals may have made better spear points), and hunted the same fallow deer and gazelles.

Neandertal genome sequence and is about to join Church's lab. In theory, one could convert a human or chimp genome to a Neandertal genome—base by base—while it is still nicely nestled in a stem cell, then clone it. But there's on the order of a million differences between the Neandertal and human genomes, and the more changes needed, the greater the risk of introducing errors.

If, somehow, a viable embryo were produced, this developing chimera would need a surrogate mother. What species would that mother belong to? Again, the obvious choice is a human, but no one knows whether a modern woman's biochemistry would be compatible with that of a Neandertal fetus. And is it ethical for a human surrogate mother to birth a Neandertal baby? Church thinks ethical views will evolve as technology improves. Once cloning works well in a variety of animals and stem cell-derived organs become commonplace, "I think the resistance to it will disappear," he says.

But others disagree. "We do not—and should not—create human beings just to satisfy our scientific curiosity," says Pääbo, pointing out that Neandertals are a species of human, so cloning them raises many of the same ethical issues as cloning a modern human.

Cloning Neandertals would involve several "ethically deplorable steps," says Briggs, including using surrogate mothers and risking high failure rates, abnormal births, and, sometimes, early death of clones. With a Neandertal, "all of these safety issues would apply, only writ large," says Wilmut. And how would a Neandertal fit into modern human society? "I see no palatable conditions," says Pääbo. "Not even for medical purposes are we thinking about creating a [modern] human being. Why would we consider something like this, which is much less pressing?"

—E.P.

"It doesn't surprise me," says archaeologist Ofer Bar-Yosef of Harvard University about the ancient DNA finding. "We always predicted low-level mixing," because some Neandertals in the Middle East, such as a female skeleton at Tabun, look less robust than Neandertals in Asia and Europe. Mixing in this region could also have happened later, when another group of modern humans came out of Africa about 60,000 years ago and perhaps met Neandertals, who were still occupying caves in the Middle East until 50,000 years ago, says Stringer.

Finally, the researchers cannot rule out the possibility that what they see as "Neandertal" motifs are really ancient genetic

Computer Kid Makes Good

Late 2007 was a real low point for Richard "Ed" Green and colleagues at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. A year earlier, in *Nature*, they had predicted that they could sequence the Neandertal genome using 20 grams of bone and 6000 runs using "next generation" sequencing technologies. They knew going in that most of the DNA in fossil bone is bacterial, with only a small percentage of Neandertal DNA. But it turned out that the bones to be sequenced had far less Neandertal DNA than the sample on which they based their projections. "We were in kind of an awkward situation of having announced to the world we were going to do it, and we were left with no concrete plan of how to do it," Green recalls. "That was very scary." Their fears increased when they discovered that their first million bases of Neandertal sequence were contaminated with modern human DNA.

"But we worked it out along the way," says Green, the postdoctoral fellow in charge of the project. He and colleagues developed methods to control contamination by putting bar codes on all DNA coming from the fossils (*Science*, 13 February 2009, p. 866). They cut down on the amount of DNA to be deciphered by cutting up much of the bacterial DNA so that the sequencing reactions ignored it. Everyone, especially Green, stresses the team effort involved. "Many people here have been able to say they 'saved the Neandertal genome project,'" he notes. And yet Green, 37, still stands out.

"Ed brought the quantitative and algorithmic horsepower needed to interpret the Neandertal data," says David Haussler of the University of California (UC), Santa Cruz, where Green now works as an assistant professor. "He invented new analysis methods that allowed the Neandertal project to happen."

That computational horsepower is what landed Green the job of shepherding the Neandertal genome. After getting a degree in computational biology from UC Berkeley, he joined Svante Pääbo's lab at the Max Planck institute in 2005 to explore

Problem solver. Richard Green overcame obstacles to sequencing Neandertal DNA.

the evolution of genes that can code for more than one protein. Pääbo and the sequencing company 454 Life Sciences in Branford, Connecticut, had just sequenced cave bear and mammoth DNA and were puzzling over the results: There was so much microbial sequence, it was hard to detect mammalian DNA. Green knew what to do: He enlisted a cluster of computers to compare the DNA with that of known sequences, including dog and elephant, so he could discard the microbial sequence and focus on the tiny bit of mammalian DNA.

"This was really the first large-scale snapshot of what the universe of [ancient] DNA looked like when it came out of a bone," Green recalls. "Then Svante said, 'Let's try Neandertal.' It was obvious that this was a once-in-a-lifetime opportunity."

He took charge of the bioinformatics effort, writing software to better detect Neandertal DNA and to deal with degradation. "He is able to design ways to analyze a whole genome under circumstances that are nonstandard," says Pääbo.

Green also coordinated the design and logistics of the rest of the project, which involved about 50 people. He was "very patient in terms of helping and training others," says Pääbo. Former graduate student Adrian Briggs agrees: "Without Ed's enthusiasm and competence, the project would never have proceeded so fast." The job required long hours at the lab, but Green says he didn't mind because the Max Planck facilities were "maximally comfortable," complete with Ping-Pong table, sauna, barbecue grill, and even a resting room.

Switching gears was not new to Green, who had started off in developmental biology as an undergrad and studied cancer biology in grad school before moving to computational biology. Now that he's settled in at UC Santa Cruz, Green expects to switch gears again. He wants to look at gene expression in nonmodel organisms while continuing to work with Pääbo on Neandertal DNA. "Ed is an incredibly skilled bioinformatician," says Pääbo. "It would be great if we could continue to work together."

—ELIZABETH PENNISI





SEPARATING THEM FROM US

Some genes that differ between modern humans and Neandertals

Gene	Significance
<i>RPTN</i>	Encodes the protein repetin, expressed in skin, sweat glands, hair roots, and tongue papilli
<i>TRPM1</i>	Encodes melastatin, a protein that helps maintain skin pigmentation
<i>THADA</i>	Associated with type 2 diabetes in humans; evolutionary changes may have affected energy metabolism
<i>DYRK1A</i>	Found in an area critical for causing Down syndrome
<i>NRG3</i>	Mutations associated with schizophrenia
<i>CADPS2/AUTS2</i>	Mutations implicated in autism
<i>RUNX2 (CBRA1)</i>	Causes cleidocranial dysplasia, characterized by delayed closure of cranial sutures, malformed clavicles, bell-shaped rib cage, and dental abnormalities
<i>SPAG17</i>	Protein important for the beating of the sperm flagellum

variants that Neandertals and some modern humans inherited from a common ancestor they shared before Neandertals split off. Although all early modern populations, including in Africa, interbred, that gene flow was not complete enough to pass these Neandertal motifs to all Africans. Human populations that were more closely related to the ancestors of Neandertals carry those motifs while Africans do not, says Reich.

To date, the genomic data don't support interbreeding in the time and place when everyone most expected it: between 45,000 and about 30,000 years ago in Europe. Neandertals and moderns lived in such proximity in France, for example, that some researchers think Neandertals imitated modern stone-tool and beadmaking technologies. But such late European mixing cannot explain the current findings, in which Asians and Europeans are equally similar to Neandertals. It's still possible that Neandertals and modern humans in Europe interbred rarely and that the Neandertal genes were swamped out in a large population of modern humans, says Slatkin.

In some ways, it is surprising that there isn't more evidence of interbreeding, now that researchers know it was biologically possible. "For some reason, they didn't interbreed a lot—something was preventing them," says evolutionary geneticist Sarah Tishkoff of the University of Pennsylvania. "Was it a cultural barrier?"

Modern motifs

The Neandertal genome also gives researchers a powerful new tool to fish for genes that have evolved recently in our lineage, after we split from Neandertals. The team compared the Neandertal genome with the genomes of five diverse modern humans. They found 78

Different paths. A partial list of genes that differ between Neandertals (*left*, reconstruction from Amud Cave, Israel) and early modern humans (*right*, reconstruction from Qafzeh Cave, Israel).

new nucleotide substitutions that change the protein-coding capacity of genes and that are present in most humans today; just five genes had more than one such substitution. That's a tiny fraction of the 3 billion bases in each genome. "Only 78 substitutions in the last 300,000 years!" says Poinar. "The fact that so few changes have become fixed on the human lineage is amazing."

But the mutations they've found so far "are all very interesting, precisely because there are so few," says Pääbo, whose team is trying to identify their function. The catalog includes changes in genes that encode proteins important for wound healing, the beating of sperm flagellum, and gene transcription (see table, above). Several of these newly evolved modern human genes encode proteins expressed in the skin, sweat glands, and inner sheaths of hair roots, as well as skin pigmentation. "The fact that three of six genes carrying multiple substitutions are in skin is fascinating," says Poinar. Pääbo speculates that these changes "reflect that skin physiology has changed but how, of course, we don't know yet."

Some of those changes are likely to be neutral changes that accumulated through genetic drift, but the team also used the Neandertal data to find other evolutionary changes that were beneficial to modern humans and so rose to high frequencies in some populations. Specifically, they have identified 15 regions containing between one and 12 genes. The widest region is located on chromosome 2 and contains the gene *THADA*, a region that varies in modern humans and that has been associated

with type 2 diabetes. Changes in this gene may have affected energy metabolism in modern humans.

Other mutations appear to be in genes important in cognitive development and that, when mutated in living people, contribute to diseases such as Down syndrome, schizophrenia, and autism. One gene, *RUNX2*, is associated with a disease that leads a spectrum of developmental abnormalities, including misshapen clavicles and a bell-shaped rib cage. Suggestively, Neandertals had bell-shaped rib cages and possibly peculiar clavicles. But precisely how all these genetic differences are expressed physiologically is the next frontier. "We need to follow up. Are there regions that are functionally significant?" says Tishkoff. By 7 May, the Neandertal data should be posted on Ensembl and the UC Santa Cruz browser, so other teams can do just that, says Pääbo.

His own group is already working on such functional studies. Postdoctoral researcher Matthias Gralle is analyzing the way these recently evolved genetic differences change the way proteins are expressed. Such studies may eventually offer clues about why Neandertals went extinct—and our ancestors didn't. "The mystery isn't just why they disappeared," says paleoanthropologist Jean-Jacques Hublin of the Max Planck Institute for Evolutionary Anthropology. "It is why we were so successful that we replaced all the others." For now, researchers are delighted that this "groundbreaking" genomic work has made it possible to ask such interesting questions, says Poinar. "This is the real appeal of this project: What will the genome of the Neandertal tell us about functional differences between the two [species]," says Poinar.

—ANN GIBBONS