

What Makes us Different?

Not very much, when you look at our DNA. But those few tiny changes made all the difference in the world

By MICHAEL D. LEMONICK, ANDREA DORFMAN

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You don't have to be a biologist or an anthropologist to see how closely the great apes--gorillas, chimpanzees, bonobos and orangutans--resemble us.

Even a child can see that their bodies are pretty much the same as ours, apart from some exaggerated proportions and extra body hair. Apes have dexterous hands much like ours but unlike those of any other creature. And, most striking of all, their faces are uncannily expressive, showing a range of emotions that are eerily familiar. That's why we delight in seeing chimps wearing tuxedos, playing the drums or riding bicycles. It's why a potbellied gorilla scratching itself in the zoo reminds us of Uncle Ralph or Cousin Vinnie--and why, in a more unsettled reaction, Queen Victoria, on seeing an orangutan named Jenny at the London Zoo in 1842, declared the beast "frightful and painfully and disagreeably human."

It isn't just a superficial resemblance. Chimps, especially, not only look like us, they also share with us some human-like behaviors. They make and use tools and teach those skills to their offspring. They prey on other animals and occasionally murder each other. They have complex social hierarchies and some aspects of what anthropologists consider culture. They can't form words, but they can learn to communicate via sign language and symbols and to perform complex cognitive tasks. Scientists figured out decades ago that chimps are our nearest evolutionary cousins, roughly 98% to 99% identical to humans at the genetic level. When it comes to DNA, a human is closer to a chimp than a mouse is to a rat.

Yet tiny differences, sprinkled throughout the genome, have made all the



difference. Agriculture, language, art, music, technology and philosophy--all the achievements that make us profoundly different from chimpanzees and make a chimp in a business suit seem so deeply ridiculous--are somehow encoded within minute fractions of our genetic code. Nobody yet knows precisely where they are or how they work, but somewhere in the nuclei of our cells are handfuls of amino acids, arranged in a specific order, that endow us with the brainpower to outthink and outdo our closest relatives on the tree of life. They give us the ability to speak and write and read, to compose symphonies, paint masterpieces and delve into the molecular biology that makes us what we are.

Until recently, there was no way to unravel these crucial differences. Exactly what gives us advantages like complex brains and the ability to walk upright--and certain disadvantages, including susceptibility to a particular type of malaria, AIDS and Alzheimer's, that don't seem to afflict chimps--remained a mystery.

But that's rapidly changing. Just a year ago, geneticists announced that they had sequenced a rough draft of the chimpanzee genome, allowing the first side-by-side comparisons of human and chimpanzee DNA. Already, that research has led to important discoveries about the development of the human brain over the past few million years and possibly about our ancestors' mating behavior as well.

And sometime in the next few weeks, a team led by molecular geneticist Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology, in Leipzig, Germany, will announce an even more stunning achievement: the sequencing of a significant fraction of the genome of Neanderthals--the human-like species we picture when we hear the word caveman--who are far closer to us genetically than chimps are. And though Neanderthals became extinct tens of thousands of years ago, Pääbo is convinced he's on the way to reconstructing the entire genome of that long-lost relative, using DNA extracted, against all odds, from a 38,000-year-old bone.

Laid side by side, these three sets of genetic blueprints--plus the genomes of gorillas and other primates, which are already well on the way to being completely sequenced--will not only begin to explain precisely what makes us human but could lead to a better understanding of human diseases and how to treat them.

FIRST GLIMMERINGS

Scientists didn't need to wait for the chimp genome to begin speculating about the essential differences between humans and apes, of course. They

didn't even need to know about DNA. Much of the vitriol directed at Charles Darwin a century and a half ago came not from his ideas about evolution in general but from his insulting but logical implication that humans and the African apes are descended from a common ancestor.

As paleontologists have accumulated more and more fossils, they have compiled data on a long list of anatomical features, including body shape, bipedalism, brain size, the shape of the skull and face, the size of canine teeth, and opposable thumbs. Using comparative analyses of these attributes, along with dating that shows when various features appeared or vanished, they have constructed increasingly elaborate family trees that show the relationships between apes, ancient hominids and us. Along the way they learned, among other things, that Darwin, even with next to no actual data, was close to being right in his intuition that apes and humans are descended from a single common ancestor--and, surprisingly, that the ability to walk upright emerged millions of years before the evolution of our big brains.

But it wasn't until the 1960s that details of our physical relationship to the apes started to be understood at the level of basic biochemistry. Wayne State University scientist Morris Goodman showed, for example, that injecting a chicken with a particular blood protein from a human, a gorilla or a chimp provoked a specific immune response, whereas proteins from orangutans and gibbons produced no response at all. And by 1975, the then new science of molecular genetics had led to a landmark paper by two University of California, Berkeley, scientists, Mary-Claire King and Allan Wilson, estimating that chimps and humans share between 98% and 99% of their genetic material.

ZEROING IN ON THE GENES

Even before the chimp genome was published, researchers had begun teasing out our genetic differences. As long ago as 1998, for example, glycobiologist Ajit Varki and colleagues at the University of California, San Diego, reported that humans have an altered form of a molecule called sialic acid on the surface of their cells. This variant is coded for by a single gene, which is damaged in humans. Since sialic acids act in part as a docking site for many pathogens, like malaria and influenza, this may explain why people are more susceptible to these diseases than, say, chimpanzees are.

A few years later, a team led by Pääbo announced that the human version of a gene called FOXP2, which plays a role in our ability to develop speech and language, evolved within the past 200,000 years--after anatomically modern humans first appeared. By comparing the protein coded by the hu-

man FOXP2 gene with the same protein in various great apes and in mice, they discovered that the amino-acid sequence that makes up the human variant differs from that of the chimp in just two locations out of a total of 715--an extraordinarily small change that may nevertheless explain the emergence of all aspects of human speech, from a baby's first words to a Robin Williams monologue. And indeed, humans with a defective FOXP2 gene have trouble articulating words and understanding grammar.

Then, in 2004, a team led by Hansell Stedman of the University of Pennsylvania identified a tiny mutation in a gene on chromosome 7 that affects the production of myosin, the protein that enables muscle tissue to contract. The mutant gene prevents the expression of a myosin variant, known as MYH16, in the jaw muscles used in biting and chewing. Since the same mutation occurs in all of the modern human populations the researchers tested--but not in seven species of nonhuman primates, including chimps--the researchers suggest that lack of MYH16 made it possible for our ancestors to evolve smaller jaw muscles some 2 million years ago. That loss in muscle strength, they say, allowed the braincase and brain to grow larger. It's a controversial claim, one disputed by anthropologist C. Owen Lovejoy of Kent State University. "Brains don't expand because they were permitted to do so," he says. "They expand because they were selected"--because they conferred extra reproductive success on their owners, perhaps by allowing them to hunt more effectively than the competition.

BEYOND THE GENES

Still, the principle of gene-by-gene comparison remains a powerful one, and just a year ago geneticists got hold of a long-awaited tool for making those comparisons in bulk. Although the news was largely overshadowed by the impact of Hurricane Katrina, which hit the same week, the publication of a rough draft of the chimp genome in the journal *Nature* immediately told scientists several important things. First, they learned that overall, the sequences of base pairs that make up both species' genomes differ by 1.23%--a ringing confirmation of the 1970s estimates--and that the most striking divergence between them occurs, intriguingly, in the Y chromosome, present only in males. And when they compared the two species' proteins--the large molecules that cells construct according to blueprints embedded in the genes--they found that 29% of the proteins were identical (most of the proteins that aren't the same differ, on average, by only two amino-acid substitutions).

The genetic differences between chimps and humans, therefore, must be relatively subtle. And they can't all be due simply to a slightly different mix of genes. Even before the human genome was sequenced back in 2000,

says biologist Sean Carroll of the University of Wisconsin, Madison, "it was estimated that humans had 100,000 genes. When we got the genome, the estimate dropped to 25,000. Now we know the overall number is about 22,000, and it might even come down to 19,000."

This shockingly small number made it clear to scientists that genes alone don't dictate the differences between species; the changes, they now know, also depend on molecular switches that tell genes when and where to turn on and off. "Take the genes involved in creating the hand, the penis and the vertebrae," says Lovejoy. "These share some of the same structural genes. The pelvis is another example. Humans have a radically different pelvis from that of apes. It's like having the blueprints for two different brick houses. The bricks are the same, but the results are very different." Those molecular switches lie in the noncoding regions of the genome--once known dismissively as junk DNA but lately rechristened the dark matter of the genome. Much of the genome's dark matter is, in fact, junk--the residue of evolutionary events long forgotten and no longer relevant. But a subset of the dark matter known as functional noncoding DNA, comprising some 3% to 4% of the genome and mostly embedded within and around the genes, is crucial. "Coding regions are much easier for us to study," says Carroll, whose new book, *The Making of the Fittest: DNA and the Ultimate Forensic Record of Evolution*, delves deep into the issue. "But it may be the dark matter that governs a lot of what we actually see."

What causes changes in both the dark matter and the genes themselves as one species evolves into another is random mutation, in which individual base pairs--the "letters" of the genetic alphabet--are flipped around like a typographical error. These changes stem from errors that occur during sexual reproduction, as DNA is copied and recombined. Sometimes long strings of letters are duplicated, creating multiple copies in the offspring. Sometimes they're deleted altogether or even picked up, turned around and reinserted backward. A group led by geneticist Stephen Scherer of the Hospital for Sick Children in Toronto has identified 1,576 apparent inversions between the chimp and human genomes; more than half occurred sometime during human evolution.

When an inversion, deletion or duplication occurs in an unused portion of the genome, nothing much changes--and indeed, the human, chimp and other genomes are full of such inert stretches of DNA. When it happens in a gene or in a functional noncoding stretch, by contrast, an inversion or a duplication is often harmful. But sometimes, purely by chance, the change gives the new organism some sort of advantage that enables it to produce more offspring, thus perpetuating the change in another generation.

WHAT THE APES CAN TEACH US

A striking example of how gene duplication may have helped propel us away from our apelike origins appeared in *Science* last month. A research team led by James Sikela of the University of Colorado at Denver and Health Sciences Center, in Aurora, Colo., looked at a gene that is believed to code for a piece of protein, called DUF1220, found in areas of the brain associated with higher cognitive function. The gene comes in multiple copies in a wide range of primates--but, the scientists found, humans carry the most copies. African great apes have substantially fewer copies, and the number found in more distant kin--orangutans and Old World monkeys--drops off even more.

Another discovery, first published online by *Nature* two months ago, describes a gene that appears to play a role in human brain development. A team led by biostatistician Katherine Pollard, now at the University of California, Davis, and Sofie Salama, of U.C. Santa Cruz, used a sophisticated computer program to search the genomes of humans, chimps and other vertebrates for segments that have undergone changes at substantially accelerated rates. They eventually homed in on 49 discrete areas they dubbed human accelerated regions, or HARs.

The region that changed most dramatically from chimps to humans, known as HAR1, turns out to be part of a gene that is active in fetal brain tissue only between the seventh and 19th weeks of gestation. Although the gene's precise function is unknown, that happens to be the period when a protein called reelin helps the human cerebral cortex develop its characteristic six-layer structure. What makes the team's research especially intriguing is that all but two of the HARs lie in those enigmatic functional noncoding regions of the genome, supporting the idea that much of the difference between species happens there.

SEX WITH CHIMPS?

Comparisons of primitive genomes have also led to an astonishing, controversial and somewhat disquieting assertion about the origin of humanity. Along with several colleagues, David Reich of the Broad Institute in Cambridge, Mass., compared DNA from chimpanzees and humans with genetic material from gorillas, orangutans and macaques. Scientists have long used the average difference between genomes as a sort of evolutionary clock because more closely related species have had less time to evolve in different directions. Reich's team measured how the evolutionary clock varied across chromosomes in the different species. To their surprise, they deduced that chimps and humans split from a common ancestor no more than 6.3 million

years ago and probably less than 5.4 million years ago. If they're correct, several hominid species now considered to be among our earliest ancestors--*Sahelanthropus tchadensis* (7 million years old), *Orrorin tugenensis* (about 6 million years old) and *Ardipithecus kadabba* (5.2 to 5.7 million years old)--may have to be re-evaluated.

And that's not the most startling finding. Reich's team also found that the entire human X chromosome diverged from the chimp's X chromosome about 1.2 million years later than the other chromosomes. One plausible explanation is that chimps and humans first split but later interbred from time to time before finally going their separate evolutionary ways. That could explain why some of the most ancient fossils now considered human ancestors have such striking mixtures of chimp and human traits--some could actually have been hybrids. Or they might have simply coexisted with, or even predated, the last common ancestor of chimps and humans.

All of that depends in part on the accuracy of fossil dating and the reliability of using genetic variation as a clock. Both methods currently carry big margins of error. But the more primate genomes that geneticists can lay side by side, the more questions they will be able to answer. "We have rough sequences for humans, orangutans, chimps, macaques," says Eric Lander, director of the Broad Institute and a leader of the research team that decoded the chimpanzee genome. "But we don't have the entire gorilla genome yet. Lemurs are coming along, and so are gibbons."

DECODING NEANDERTHALS

Also coming along, thanks to two independent teams of researchers, is the genome of the closest relative of all: the Neanderthal. Ancestors of Neanderthals first appeared some 500,000 years ago, and for a long time it was a toss-up whether that lineage would outlive our own species, at least in Europe and western Asia--or whether, bizarre as it seems today, they would both survive indefinitely. The Neanderthals held out for hundreds of thousands of years. A discovery published online by *Nature* last month suggests Neanderthals may have made their last stand in Gibraltar, on the southern tip of the Iberian Peninsula, surviving until about 28,000 years ago--and possibly even longer.

The Neanderthals weren't nearly as primitive as many assume, observes Eddy Rubin, director of the Department of Energy's Joint Genome Institute in Walnut Creek, Calif. "They had fire, burial ceremonies, the rudiments of what we would call art. They were advanced--but nothing like what humans have done in the last 10,000 to 15,000 years." We eventually outcompeted them, and the key to how we did so may well lie in our genes. So two years

ago, Svante Pääbo, the man who deconstructed the FOXP2 language gene and has done considerable research on ancient DNA, launched an effort to re-create the Neanderthal genome. Rubin, meanwhile, is tackling the same task using a different technique.

The job isn't an easy one. Like any complex organic molecule, DNA degrades over time, and bones that lie in the ground for thousands of years become badly contaminated with the DNA of bacteria and fungi. Anyone who handles the fossils can also leave human DNA behind. After probing the remains of about 60 different Neanderthals out of the 400 or so known, Pääbo and his team found only two with viable material. Moreover, he estimates, only about 6% of the genetic material his team extracts from the bones turns out to be Neanderthal DNA.

As a result, progress is maddeningly slow. And while he can't reveal details, Pääbo says he'll soon be announcing in a major scientific journal the sequencing of 1 million base pairs of the Neanderthal genome. And he says he has 4 million more in the bag. Rubin, meanwhile, is also poised to publish his results, but refuses to divulge specifics. "Pääbo's team has significantly more of a sequence than we do," he says. "Some of the dates will differ, but the conclusions are largely similar."

Although Pääbo admits that he still hasn't learned much about what distinguishes us from our closest cousins, simply showing he can reconstruct significant DNA sequences from such long-dead creatures is an important proof of concept. Both he and Rubin agree that within a couple of years a reasonably complete Neanderthal genome should be available. "It will tell us about aspects of biology, like soft tissue, that we can't say anything about right now," Rubin notes. "It could tell us about disease susceptibility and immunity. And in places where the sequence overlaps that of humans, it will enable us to compare a prehistoric creature with chimps." Someday it may even be possible to insert equivalent segments of human and Neanderthal DNA into different laboratory mice in order to see what effects they produce.

WHAT IT ALL MEANS

Precisely how useful this information will be is hard to assess. Indeed, a few experts are dismissive of the whole project. "I'm not sure what Neanderthals will tell us," says Kent State's Lovejoy. "They're real late [in terms of human evolution]. And they represent, at best, a little environmental isolate in Europe. I can't imagine we're going to learn much about human evolution by studying them." Lovejoy is even more dismissive about claims that ancestors of chimps and humans interbred, arguing that using muta-

tion rates in the genome to time evolutionary changes is extraordinarily imprecise.

In fact, even the most ardent proponents of genome-comparison research acknowledge that pretty much everything we know so far is preliminary. "We're interested in traits that really distance us from other organisms," says Wisconsin's Carroll,



"such as susceptibility to diseases, big brains, speech, walking upright, opposable thumbs. Based on the biology of other organisms, we have to believe that those are very complex traits. The development of form, the increase in brain size, took place over a long period of time, maybe 50,000 generations. It's a pretty complicated genetic recipe."

But even the toughest critics acknowledge that these studies have enormous potential. "We will eventually be able to pinpoint every difference between every animal on the planet," says Lovejoy. "And every time you throw another genome, like the gorilla's, into the mix, you increase the chances even more."

Some of the differences could have enormous practical consequences. Since his discovery that human cells lack one specific form of sialic acid, which was accomplished even before the human genome was decoded, Varki and his collaborators have determined that 10 of the 60 or so genes that govern sialic-acid biology show major differences between chimps and humans. "And in every case," says Varki, "it's humans who are the odd one out." Such revelations could probably lead to a better understanding of such devastating diseases as malaria, AIDS and viral hepatitis and likely do so faster than by studying the human genome alone.

For most of us, though, it's the grand question about what it is that makes us human that renders comparative genome studies so compelling. As scientists keep reminding us, evolution is a random process in which haphazard genetic changes interact with random environmental conditions to produce an organism somehow fitter than its fellows. After 3.5 billion years of such randomness, a creature emerged that could ponder its own origins-- and revel in a Mozart adagio. Within a few short years, we may finally understand precisely when and how that happened.